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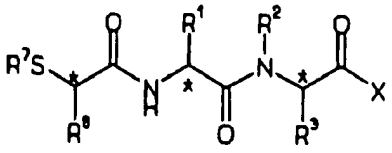
(54) Title: PEPTIDYL COMPOUNDS AND THEIR THERAPEUTIC USE AS INHIBITORS OF METALLOPROTEASES

(57) Abstract

Compounds of general formula (I) have utility as inhibitors of matrix metalloproteinases and TNF.



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LACTAM-CONTAINING HYDROXAMIC ACID DERIVATIVES, THEIR PREPARATION AND THEIR USE AS INHIBITORS OF MATRIX METALLOPROTEASE

TECHNICAL FIELD

This invention is directed to compounds which are useful in treating diseases associated with excess and/or unwanted matrix metalloprotease activity, particularly collagenase and/or stromelysin activity. More specifically, the invention is directed to hydroxamic acid compounds that contain a substituted lactam ring.

BACKGROUND

A number of enzymes effect the breakdown of structural proteins and are structurally related metalloproteases. These include human skin fibroblast collagenase, human skin fibroblast gelatinase, human sputum collagenase and gelatinase, and human stromelysin. These are zinc-containing metalloprotease enzymes, as are the angiotensin-converting enzymes and the enkephalinases. Collagenase, stromelysin and related enzymes are important in mediating the symptomatology of a number of diseases, including rheumatoid arthritis (Mullins, D. E., et al., Biochim Biophys Acta (1983) 695:117-214); osteoarthritis (Henderson, B., et al., Drugs of the Future (1990) 15:495-508); the metastasis of tumor cells (ibid, Broadhurst, M. J., et al., European Patent Application 276,436 (published 1987), Reich, R., et al., 48 Cancer Res 3307-3312 (1988); and various ulcerated conditions. Ulcerative conditions can result in the cornea as the result of alkali burns or as a result of infection by Pseudomonas aeruginosa, Acanthamoeba, Herpes simplex and vaccinia viruses.

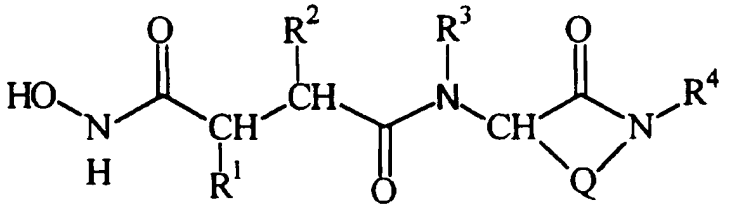
Other conditions characterized by unwanted matrix metalloprotease activity include periodontal disease, epidermolysis bullosa and scleritis. In view of the involvement of matrix metalloproteases in a number of disease conditions, attempts have been made to prepare inhibitors to these enzymes. A number of such inhibitors are disclosed in the literature. Examples include U.S. Patent No. 5,183,900, issued February 2, 1993 to Galardy; U.S. Patent No. 4,996,358, issued February 26, 1991 to Handa, et al.; U.S. Patent No. 4,771,038, issued September 13, 1988 to Wolanin, et al.; U.S. Patent Number 4,743,587, issued May 10, 1988 to Dickens, et al., European Patent Publication Number 575,844, published December 29, 1993 by Broadhurst, et al.; International Patent Publication No. WO 93/09090, published May 13, 1993 by Isomura, et al.; World Patent Publication 92/17460, published October 15, 1992 by

Markwell et al.; and European Patent Publication Number 498,665, published August 12, 1992 by Beckett, et al.

It is well known in the art that inhibitors of matrix metalloproteases are useful in treating diseases caused, at least in part, by breakdown of structural proteins. Though a variety of inhibitors have been prepared, there is a continuing need for potent matrix metalloprotease inhibitors useful in treating such diseases. Applicants have found that, surprisingly, the lactam-containing hydroxamic acids of the present invention are potent inhibitors of collagenase and/or stromelysin. The compounds of the present invention therefore may be useful for the treatment of conditions and diseases which are characterized by unwanted activity by the class of proteins which destroy structural proteins.

SUMMARY OF THE INVENTION

The invention provides compounds which are useful as inhibitors of matrix metalloproteases, and which are effective in treating conditions characterized by excess activity of these enzymes. In particular, the present invention relates to a compound having a structure according to Formula (I)



(I)

wherein

(A) (1) (a) R^1 is hydrogen; alkyl; heteroalkyl; alkenyl; a heterocyclic ring; a carbocyclic ring; alkoxy; carbocycle-alkyl; heterocycle-alkyl; carbocycle-heteroalkyl; or heterocycle-heteroalkyl; and

(b) R^2 is hydrogen; hydroxy; alkyl; alkenyl; alkynyl; heteroalkyl; a heterocyclic ring; a carbocyclic ring; carbocycle-alkyl; heterocycle-alkyl; or -OR, where R is alkyl, alkenyl, or carbocycle-alkyl; or

(2) R^1 and R^2 together form a cycloalkyl ring having from 3 to 8 ring atoms;

(B) R^3 is hydrogen; alkyl; or carbocycle-alkyl;

(C) R^4 is

(1) alkyl;

(2) carbocycle-alkyl;

(3) $-X-C(=Y)-Z-R^5$ or $-X-CH_2-Z-R^5$, where

(a) X is covalent bond or alkyl;

(b) Y is O, S, or NH;

(c) Z is O, S, or NH; and

(d) R^5 is hydrogen; alkyl; alkenyl; carbocycle-alkyl; or aryl; or

(4) $-SO_2-R^6$, where R^6 is alkyl, carbocylce-alkyl, heterocycle-alkyl, or aryl; and

(D) Q is an alkyl chain, an alkenyl chain, a heteroalkyl chain, or a heteroalkenyl chain; wherein said chain has 2, 3, or 4 chain atoms and is unsubstituted or substituted with one or more alkyl moieties;

or a pharmaceutically-acceptable salt, or biohydrolyzable alkoxyamide, acyloxyamide, or imide thereof.

These compounds have the ability to inhibit at least one mammalian matrix metalloprotease. Accordingly, in other aspects, the invention is directed to pharmaceutical compositions containing the compounds of Formula (I), and to methods of treating diseases characterized by matrix metalloprotease activity using these compounds or the pharmaceutical compositions containing them.

Matrix metalloproteases at a particularly undesired location can be targeted by conjugating the compounds of the invention to a targeting ligand specific for a marker at that location such as an antibody or fragment thereof or a receptor ligand.

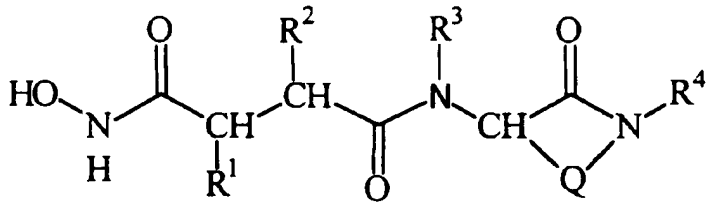
The invention is also directed to various other processes which take advantage of the unique properties of these compounds. Thus, in another aspect, the invention is directed to the compounds of Formula (I) conjugated to solid supports. These conjugates can be used as affinity reagents for the purification of a desired matrix metalloprotease.

In another aspect, the invention is directed to the compounds of Formula (I) conjugated to label. As the compounds of the invention bind to at least one matrix metalloprotease, the label can be used to detect the presence of relatively high levels of matrix metalloprotease in vivo or in vitro cell culture.

In addition, the compounds of Formula (I) can be conjugated to carriers which permit the use of these compounds in immunization protocols to prepare antibodies specifically immunoreactive with the compounds of the invention. These antibodies are then useful both in therapy and in monitoring the dosage of the inhibitors.

DETAILED DESCRIPTION

The compounds of the present invention are inhibitors of mammalian matrix metalloproteases. Preferably, the compounds are those of Formula (I) where the Q-containing heterocycle has one nitrogen atom. These compounds are the following



(I)

wherein

(A) (1) (a) R^1 is hydrogen; alkyl; heteroalkyl; alkenyl; a heterocyclic ring; a carbocyclic ring; alkoxy; carbocycle-alkyl; heterocycle-alkyl; carbocycle-heteroalkyl; or heterocycle-heteroalkyl; and

(b) R^2 is hydrogen; hydroxy; alkyl; alkenyl; alkynyl; heteroalkyl; a heterocyclic ring; a carbocyclic ring; carbocycle-alkyl; heterocycle-alkyl; or -OR, where R is alkyl, alkenyl, or carbocycle-alkyl; or

(2) R^1 and R^2 together form a cycloalkyl ring having from 3 to 8 ring atoms;

(B) R^3 is hydrogen; alkyl; or carbocycle-alkyl;

(C) R^4 is

(1) alkyl;

(2) carbocycle-alkyl;

(3) $-X-C(=Y)-Z-R^5$ or $-X-CH_2-Z-R^5$, where

(a) X is covalent bond or alkyl;

(b) Y is O, S, or NH;

(c) Z is O, S, or NH; and

(d) R^5 is hydrogen; alkyl; alkenyl; carbocycle-alkyl; or aryl; or

(4) $-SO_2-R^6$, where R^6 is alkyl, carbocylce-alkyl, heterocycle-alkyl, or aryl; and

(D) Q is $-[-C(R^7)_2-]-_n$, where

(1) n is the integer 2, 3, or 4; and

(2) each R^7 is independently hydrogen or alkyl so the Q-containing heterocycle is saturated; or the R^7 moiety on two adjacent carbon

atoms is a covalent bond such that the Q-containing heterocycle in Formula (I) is unsaturated;

or a pharmaceutically-acceptable salt, or biohydrolyzable alkoxyamide, acyloxyamide, or imide thereof.

Definitions and Usage of Terms:

The following is a list of definitions for terms used herein.

"Acyl" or "carbonyl" is a radical formed by removal of the hydroxy from a carboxylic acid (i.e., R-C(=O)-). Preferred acyl groups include (for example) acetyl, formyl, and propionyl.

"Acyloxy" is an oxygen radical having an acyl substituent (i.e., -O-acyl); for example,-O-C(=O)-alkyl.

"Acylamino" is an amino radical having an acyl substituent (i.e., -N-acyl); for example, -NH-C(=O)-alkyl.

"Alkoxyacyl" is an acyl radical (-C(=O)-) having an alkoxy subtituent (i.e., -O-R), for example, -C(=O)-O-alkyl.

"Alkenyl" is an unsubstituted or substituted hydrocarbon chain radical having 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 carbon atoms; preferably from 2 to 10 carbon atoms; more preferably from 2 to 8; except where indicated. Alkenyl substituents have at least one olefinic double bond (including, for example, vinyl, allyl and butenyl).

"Alkynyl" is an unsubstituted or substituted hydrocarbon chain radical having 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 carbon atoms; preferably from 2 to 10 carbon atoms; more preferably from 2 to 8; except where indicated. The chain has at least one carbon-carbon triple bond.

"Alkoxy" is an oxygen radical having a hydrocarbon chain substituent, where the hydrocarbon chain is an alkyl or alkenyl (i.e., -O-alkyl or -O-alkenyl). Preferred alkoxy groups include (for example) methoxy, ethoxy, propoxy and allyloxy.

"Alkoxyalkyl" is an unsubstituted or substituted alkyl moiety substituted with an alkoxy moiety (i.e., -alkyl-O-alkyl). Preferred is where the alkyl has 1, 2, 3, 4, 5 or 6 carbon atoms (more preferably 1 to 3 carbon atoms), and the alkyoxy has 1, 2, 3, 4, 5 or 6 carbon atoms (more preferably 1 to 3 carbon atoms).

"Alkyl" is an unsubstituted or substituted saturated hydrocarbon chain radical having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 carbon atoms; preferably from 1 to 10 carbon atoms; more preferably 1 to 4; except where indicated. Preferred alkyl groups include (for example) substituted or unsubstituted methyl, ethyl, propyl, is pr pyl, and butyl.

"Alkylamino" is an amino radical having one (secondary amine) or two (tertiary amine) alkyl substituents (i.e., -N-alkyl). For example, methylamine ($-NHCH_3$), dimethylamine ($-N(CH_3)_2$), methylethylamine ($-N(CH_3)CH_2CH_3$).

"Aminoacyl" is acyl radical having an amino substituent (i.e., -C(=O)-N); for example, $-C(=O)-NH_2$. The amino group of the aminoacyl moiety may be unsubstituted (i.e., primary amine) or may be substituted with one (secondary amine) or two (i.e., tertiary amine) alkyl groups.

"Aryl" is an aromatic carbocyclic ring radical. Preferred aryl groups include (for example) phenyl, tolyl, xylyl, cumenyl and naphthyl.

"Arylalkyl" is an alkyl radical substituted with an aryl group. Preferred arylalkyl groups include benzyl, phenylethyl, and phenylpropyl.

"Arylalkylamino" is an amine radical substituted with an arylalkyl group (e.g., -NH-benzyl).

"Arylamino" is an amine radical substituted with an aryl group (i.e., -NH-aryl).

"Aryloxy" is an oxygen radical having an aryl substituent (i.e., -O-aryl).

"Carbocyclic ring" is an unsubstituted or substituted, saturated, unsaturated or aromatic, hydrocarbon ring radical. Carbocyclic rings are monocyclic or are fused, bridged or spiro polycyclic ring systems. Monocyclic carbocyclic rings generally contain 3, 4, 5, 6, 7, 8 or 9 atoms, preferably 3 to 6 atoms. Polycyclic carbocyclic rings contain 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 atoms, preferably from 7 to 13 atoms.

"Carbocycle-alkyl" is an unsubstituted or substituted alkyl radical substituted with a carbocyclic ring. Unless otherwise specified, the carbocyclic ring is preferably an aryl or cycloalkyl; more preferably an aryl. Preferred carbocycle-alkyl groups include benzyl, phenylethyl and phenylpropyl.

"Carbocycle-heteroalkyl" is an unsubstituted or substituted heteroalkyl radical substituted with a carbocyclic ring. Unless otherwise specified, the carbocyclic ring is preferably an aryl or cycloalkyl; more preferably an aryl. The heteroalkyl is preferably 2-oxa-propyl, 2-oxa-ethyl, 2-thia-propyl, or 2-thia-ethyl.

"Carboxyalkyl" is an unsubstituted or substituted alkyl radical substituted with with a carboxy (-C(=O)OH) moiety. For example, $-CH_2-C(=O)OH$.

"Cycloalkyl" is a saturated carbocyclic ring radical. Preferred cycloalkyl groups include (for example) cyclopropyl, cyclobutyl and cyclohexyl.

"Cycloheteroalkyl" is a saturated heterocyclic ring. Preferred cycloheteroalkyl groups include (for example) morph line, piperadine, and piperazine.

"Fused rings" are rings that are superimposed together such that they share two ring atoms. A given ring may be fused to more than one other ring.

"Heterocycle-alkyl" is an alkyl radical substituted with a heterocyclic ring. The heterocyclic ring is preferably an heteroaryl or cycloheteroalkyl; more preferably an heteroaryl.

"Heterocycle-heteroalkyl" is an unsubstituted or substituted heteroalkyl radical substituted with a heterocyclic ring. The heterocyclic ring is preferably an aryl or cycloheteroalkyl; more preferably an aryl.

"Heteroatom" is a nitrogen, sulfur or oxygen atom. Groups containing one or more heteroatoms may contain different heteroatoms.

"Heteroalkenyl" is an unsubstituted or substituted unsaturated chain radical having 3, 4, 5, 6, 7 or 8 members comprising carbon atoms and one or two heteroatoms. The chain has at least one carbon-carbon double bond.

"Heteroalkyl" is an unsubstituted or substituted saturated chain radical having 2, 3, 4, 5, 6, 7 or 8 comprising carbon atoms and one or two heteroatoms.

"Heterocyclic ring" is an unsubstituted or substituted, saturated, unsaturated or aromatic ring radical comprised of carbon atoms and one or more heteroatoms in the ring. Heterocyclic rings are monocyclic or are fused, bridged or spiro polycyclic ring systems. Monocyclic heterocyclic rings contain 3, 4, 5, 6, 7, 8 or 9 atoms, preferably 4 to 7 atoms. Polycyclic rings contain 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 atoms, preferably from 7 to 13 atoms.

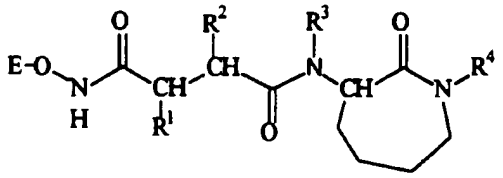
"Heteroaryl" is an aromatic heterocyclic ring radical. Preferred heteroaryl groups include (for example) thienyl, furyl, pyrrolyl, pyridinyl, pyrazinyl, thiazolyl, pyrimidinyl, quinolinyl, and tetrazolyl.

"Halo", "halogen", or "halide" is a chloro, bromo, fluoro or iodo atom radical. Chloro and fluoro are preferred halides.

Also, as referred to herein, a "lower" hydrocarbon moiety (e.g., "lower" alkyl) is a hydrocarbon chain comprised of 1, 2, 3, 4, 5 or 6, preferably from 1 to 4, carbon atoms.

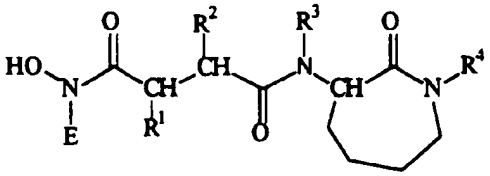
A "pharmaceutically-acceptable salt" is a cationic salt formed at any acidic (e.g., carboxyl) group, or an anionic salt formed at any basic (e.g., amino) group. Many such salts are known in the art, as described in World Patent Publication 87/05297, Johnston et al., published September 11, 1987 (incorporated by reference herein). Preferred cationic salts include the alkali metal salts (such as sodium and potassium), and alkaline earth metal salts (such as magnesium and calcium). Pr ferred anionic salts include the halides (such as chloride salts).

"Biohydrolyzable alkoxyamide" and "Biohydrolyzable acyloxyamide" are amides of a hydroxamic acid that do not essentially interfere with the inhibitory activity of the compound, or that are readily converted in vivo by a human or lower animal subject to yield an active hydroxamic acid. A biohydrolyzable alkoxyamide derivative of the Formula (I) compounds is represented by the following:



where E is an alkyl moiety. A biohydrolyzable acyloxyamide derivative of the Formula (I) compounds is where E is an acyl moiety (e.g. R-C(=O)-).

A "biohydrolyzable hydroxy imide" is an imide of a Formula (I) compound that does not interfere with the metalloprotease inhibitory activity of these compounds, or that is readily converted in vivo by a human or lower animal subject to yield an active Formula (I) compound. Such hydroxy imides include those that do not interfere with the biological activity of the Formula (I) compounds. These imides have a structure according to the following:



where E is an acyl moiety (e.g., -C(=O)-R).

A "solvate" is a complex formed by the combination of a solute (e.g., a hydroxamic acid) and a solvent (e.g., water). See J. Honig et al., The Van Nostrand Chemist's Dictionary, p. 650 (1953). Pharmaceutically-acceptable solvents used according to this invention include those that do not interfere with the biological activity of the hydroxamic acid (e.g., water, ethanol, acetic acid, N,N-dimethylformamide).

The illustration of specific protected forms and other derivatives of the Formula (I) compounds is not intended to be limiting. The application of other useful protecting groups, salt forms, etc. is within the ability of the skilled artisan.

As defined above and as used herein, substituent groups may themselves be substituted. Such substitution may be with one or more substituents. Such substituents include those listed in C. Hansch and A. Leo, Substituent Constants for

Correlation Analysis in Chemistry and Biology (1979), incorporated by reference herein. Preferred substituents include (for example) alkyl, alkenyl, alkoxy, hydroxy, oxo, nitro, amino, aminoalkyl (e.g., aminomethyl, etc.), cyano, halo, carboxy, alkoxyaceyl (e.g., carboethoxy, etc.), thiol, aryl, cycloalkyl, heteroaryl, heterocycloalkyl (e.g., piperidinyl, morpholinyl, pyrrolidinyl, etc.), imino, thioxo, hydroxyalkyl, aryloxy, arylalkyl, and combinations thereof.

As used herein, "mammalian matrix metalloprotease" means any metal-containing enzyme found in mammalian sources which is capable of catalyzing the breakdown of collagen, gelatin or proteoglycan under suitable assay conditions. Appropriate assay conditions can be found, for example, in U.S. Pat. No. 4,743,587, which references the procedure of Cawston, et al., Anal Biochem (1979) 99:340-345, use of a synthetic substrate is described by Weingarten, H., et al., Biochem Biophy Res Comm (1984) 139:1184-1187. Any standard method for analyzing the breakdown of these structural proteins can, of course, be used. The matrix metalloprotease enzymes referred to herein are all zinc-containing proteases which are similar in structure to, for example, human stromelysin or skin fibroblast collagenase. The ability of candidate compounds to inhibit matrix metalloprotease activity can, of course, be tested in the assays described above. Isolated matrix metalloprotease enzymes can be used to confirm the inhibiting activity of the invention compounds, or crude extracts which contain the range of enzymes capable of tissue breakdown can be used.

Compounds:

Referring to Formula (I), the R^1 substituent group is selected from hydrogen; alkyl; heteroalkyl; alkenyl; a heterocyclic ring; a carbocyclic ring; alkoxy; carbocycle-alkyl; heterocycle-alkyl; carbocycle-heteroalkyl; and heterocycle-heteroalkyl. Preferred is where R^1 is hydrogen; alkyl; alkenyl; a heterocyclic ring; alkoxy; carbocycle-alkyl; or aminoalkyl. More preferred is where R^1 is hydrogen; C_1-C_8 alkyl; aminoalkyl; or benzyl. Most preferred is where R^1 is hydrogen, methyl, ethyl, or propyl.

R^2 is selected from hydrogen; hydroxy; alkyl; alkenyl; alkynyl; heteroalkyl; a heterocyclic ring; a carbocyclic ring; carbocycle-alkyl; heterocycle-alkyl; and -OR, where R is alkyl, alkenyl, or carbocycle-alkyl. Preferred is where R^2 is hydrogen; alkyl; or aminoalkyl. More preferred is where R^2 is hydrogen or C_1-C_8 alkyl. Particularly preferred is where R^2 is n-octyl, n-pentyl or 2-methylpropyl.

In the alternative, R^1 and R^2 can together form a cycloalkyl ring having from 3 to 8 ring atoms; preferably 5 to 7 ring atoms; more preferably 6 atoms. Preferred is where R^1 and R^2 do not combine to form a ring.

R^3 is selected from hydrogen; alkyl; and carbocycle-alkyl (more preferably C_1-C_2 alkyl). Preferred is where R^3 is hydrogen.

R^4 is selected from alkyl; carbocycle-alkyl; alkoxyalkyl; -X-C(=Y)-Z-R^5 or -X-CH_2-Z-R^5, where (a) X is covalent bond or alkyl; (b) Y is O, S, or NH; (c) Z is O, S, or NH; R^5 is hydrogen; alkyl, alkenyl, carbocycle-alkyl, or aryl.

When R^4 is alkyl, preferred is C_1-C_8 alkyl.

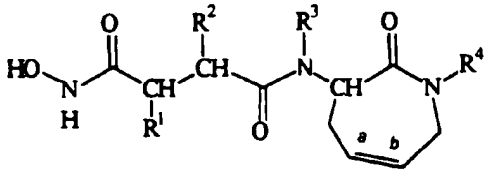
When R^4 is -X-C(=Y)-Z-R^5, X is preferably C_1-C_3 alkyl (more preferably C_1-C_2 alkyl), Y is preferably O, and Z is preferably NH or O. When Y and Z are both O, R^5 is preferably alkyl (preferably methyl or ethyl; most preferably methyl) or carbocycle-alkyl (preferably benzyl); most preferably alkyl. When Y is O and Z is NH, R^5 is preferably alkyl or carbocycle-alkyl; more preferably methyl, ethyl, butyl, or benzyl.

When R^4 is -X-CH_2-Z-R^5, X is preferably C_1-C_3 alkyl, Z is preferably O or S, and R^5 is preferaby alkyl or carbocycle-alkyl (more preferably alkyl). Particularly preferred is where X is C_1, Z is O and R^5 is C_1-C_3 alkyl.

When R^4 is -SO_2R^6, R^6 is alkyl, carbocylce-alkyl, heterocycle-alkyl, or aryl; preferably aryl (preferably phenyl; most preferably 4-methylphenyl).

As indicated above, particularly preferred compounds of the present invention are those where the Q-containing heterocycle has only one ring nitrogen atom. That is, where Q is -[-$C(R^7)_2$-]-$_n$, where n is the integer 2, 3, or 4 (more preferably 3 or 4). Particurlary preferred is where n is 4, such that the Q-containing heterocycle has 7 ring atoms. Each R^7 is independently hydrogen or alkyl; or the R^7 moiety on two adjacent carbon atoms is a covalent bond such that the Q-containing heterocycle in Formula (I) is unsaturated. Preferred compounds are those where the Q-containing heterocycle is saturated; most preferably where each R^7 is hydrogen.

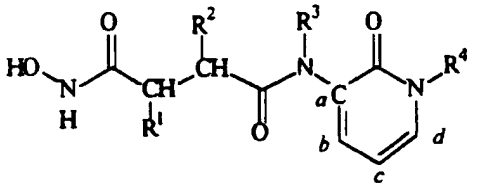
The following illustrates compounds where the Q-containing heterocycle is unsaturated:



In this structure, the heterocycle has seven members (i.e., n=4). Referring to Formula (I), carbon atoms *a* and *b* each represent a -$C(R^7)_2$- moiety where one R^7 is hydrogen and the other is a covalent bond, such that a double bond exists between atoms *a* and *b*.

Two groups of adjacent carbon atoms may have R^7 moi ties that are covalent bonds, such that the lactam ring has two points of unsaturation (i.e., two double bonds).

The following illustrates such rings where the Q-containing heterocylce has two points of unsaturation:



In this structure, the heterocycle has six members (i.e., n = 3). Referring to Formula (I), carbon atoms *a* and *b* each represent a $-C(R^7)_2-$ moiety where one R^7 is hydrogen and the other is a covalent bond, such that a double bond exists between atoms *a* and *b*. In addition, *c* and *d* each represents a $-C(R^7)_2-$ moiety where one R^7 is hydrogen and the other is a covalent bond, such that a double bond exists between atoms *c* and *d*.

The following table lists representative preferred compounds within the scope of the invention. The table is not intended to be an exhaustive list of the compounds within the scope of the invention. Referring to Formula (I), Q is $(-CH_2-)_n$, n is 4, and R^3 is hydrogen in each instance.

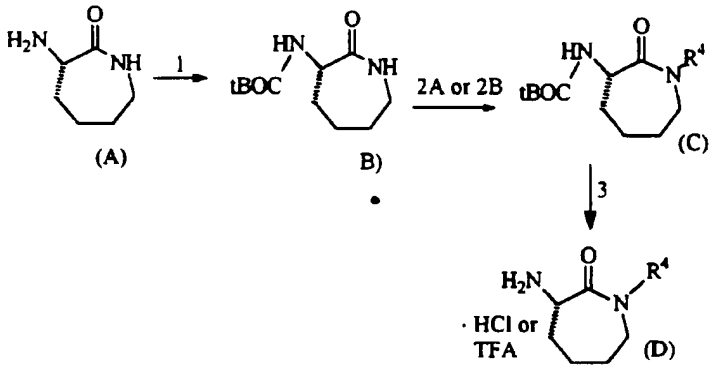
Cmp.	R^1	R^2	R^4
1	hydrogen	2-methylpropyl	$-CH_2-C(=O)-O-CH_3$
2	hydrogen	2-methylpropyl	$-CH_2-C(=O)-NH-CH_3$
3	hydrogen	2-methylpropyl	$-CH_2-C(=O)-O-C(CH_3)_3$
4	hydrogen	2-methylpropyl	$-CH_2$-phenyl
5	hydrogen	2-methylpropyl	$-CH_2-C(=O)-O-CH_2$-phenyl
6	hydrogen	2-methylpropyl	$-CH_2-C(=O)-NH-CH_2$-phenyl
7	hydrogen	2-methylpropyl	$-CH_2-C(=O)-O-CH_3$
8	hydrogen	$-(CH_2)_4CH_3$	$-CH_2-C(=O)-NH-(CH_2)_3-CH_3$
9	hydrogen	$-(CH_2)_7CH_3$	$-CH_2-C(=O)-O-CH_3$
10	hydrogen	$-(CH_2)_7CH_3$	$-SO_2$-phenyl
11	hydrogen	$-(CH_2)_7CH_3$	$-CH_2-CH_2-O-CH_3$
12	hydrogen	$-(CH_2)_7CH_3$	$-(CH_2)_3CH_3$
13	CH_3 (S form)	2-methylpropyl	$-CH_2-C(=O)-O-CH_3$
14	CH_3 (R form)	2-methylpropyl	$-CH_2-C(=O)-O-CH_3$
15	$CH_3CH_2CH_2-$	2-methylpropyl	$-CH_2-C(=O)-O-CH_3$
16	$-(CH_2)_2-CH_2OH$ (S form)	2-methylpropyl	$-CH_2-C(=O)-O-CH_3$
17	CH_3 (S form)	$-(CH_2)_7CH_3$	$-CH_2-C(=O)-O-CH_3$
18	CH_3 (R form)	$-(CH_2)_7CH_3$	$-CH_2-C(=O)-O-CH_3$
19	CH_3 (S form)	$-(CH_2)_7CH_3$	$-CH_2-CH_2-O-CH_3$

20	CH_3 (R form)	$-(CH_2)_7CH_3$	$-CH_2-CH_2-O-CH_3$

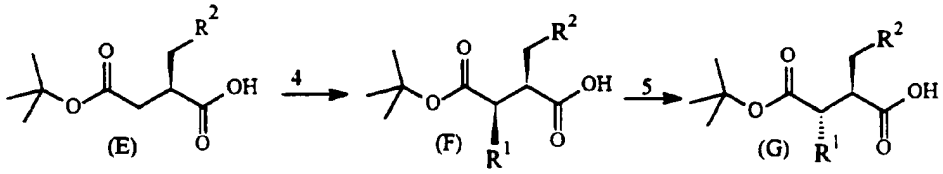
General Schemes for Compound Preparation:

The hydroxamic compounds of Formula (I) can be prepared using a variety of procedures. General schemes include the following. (Representative examples are described for making specific compounds hereinbelow.)

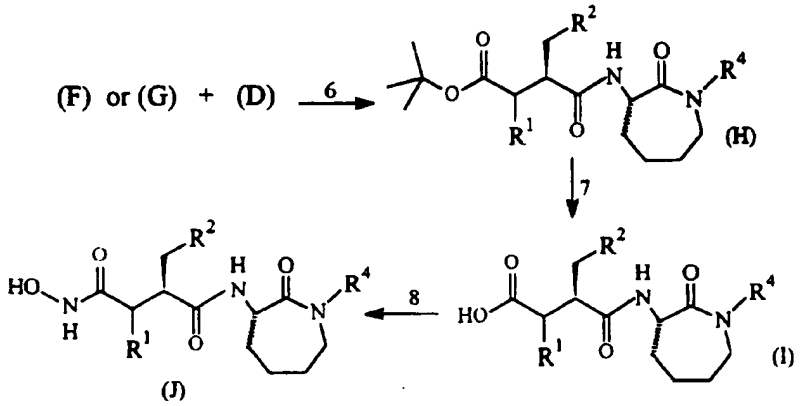
a. General Scheme 1:



1) $(BOC)_2O$, DMSO; 2A) $LiN(TMS)_2$; R^4-X, THF;
2B) t-BuOK, R^4-X, DMF; 3) TFA/CH_2Cl_2 or HCl/Et_2O



4) $LiN(TMS)_2$, THF, R^1-X; 5) LDA, THF



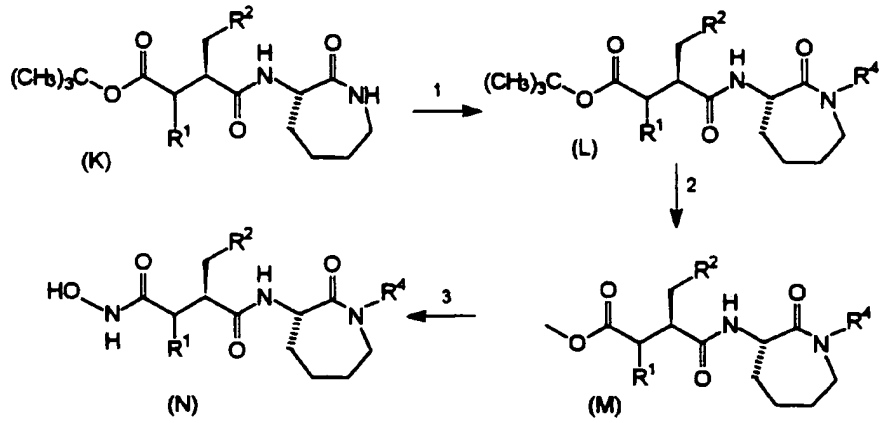
6) EDAC, HOBT, NMM, DMF, Odeg. C; 7) TFA, CH_2Cl_2;
8A-i)EDAC, HOBT, NMM, DMF, $BnONH_2$·HCl, O deg. C; ii) H_2/Pd-C, EtOH;
8B-i) CH_2N_2; ii) NH_2OH·HCl/KOH, MeOH

Commercially available Caprolactam (A) is protected to give (B), followed by alkylation of the amydic nitrogen under appropriate conditions to give (C). The derivatized lactam (C) is deprotected under acidic conditions to give the amine salt (D) which is then used for coupling to various succinates as described in Scheme 2 and 3.

The various alkyl succinates (E) are synthesized following Evan's chiral alkylation method (D. A. Evans, et al., Org. Synth. Vol. 86, p 83 (1990), incorporated herein by reference). The dianion generated by treating (E) with a hindered base is alkylated to give syn-disubstituted succinates (F) which on further treatment with LDA gives the desired anti-diastereomer (G) in reasonable yield (H. J. Crimmin, et al., Synlett, 137-138 (1993)).

The acid (F) or (G) and the amine salt (D) are coupled under a mild condition to give the amide (H) (depicted without specifying stereochemistry), which on deprotection under acidic conditions gives the corresponding acid (I). A final transformation is carried out to convert the purified acid to the desired inhibitor (J).

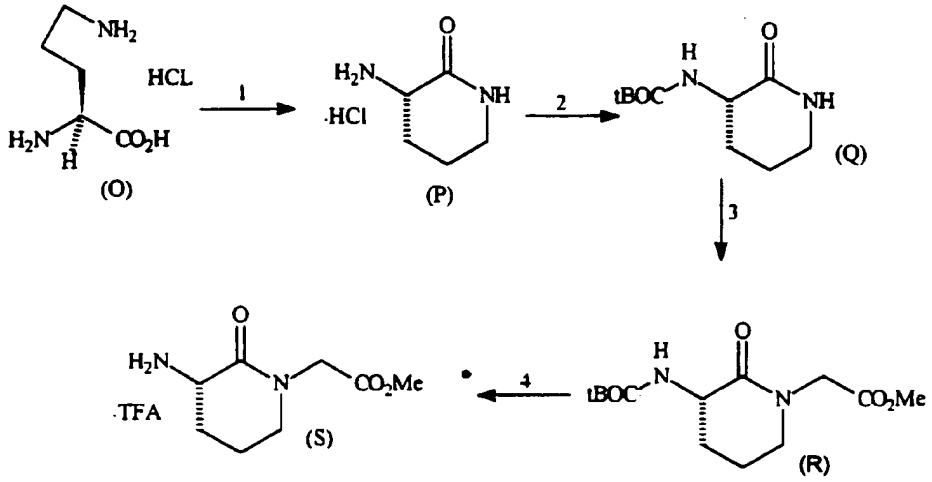
b. General Scheme 2:



A direct alkylation method can also be utilized to synthesize the final inhibitors. For example, the treatment of intermediate (K) (prepared by reacting Compound A with Compound F or G according to Scheme 1) with a hindered base at low temperature followed by a quench of the anion with an alkylating agent gives (L) which on deprotection under acidic conditions and re-esterification provides (M) in good yield. A direct treatment of this ester with freshly-generated hydroxylamine then produces the final inhibitor (N).

c. General Scheme 3:

The compounds of the present invention having a lactam ring with 5 or 6 members can be prepared as follows.

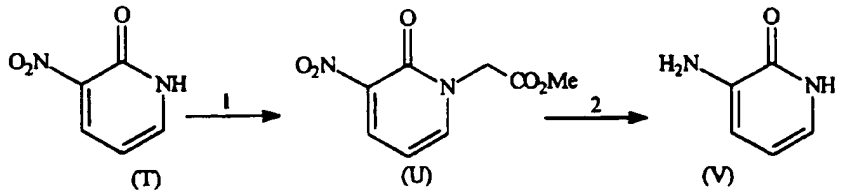


1) $(TMS)_2NH$, CH_3CN; 2) $(BOC)_2O$; DMSO
3) $LiN(TMS)_2$, THF, $BrCH_2COOMe$; 4) TFA, CH_2Cl_2

For example, L-ornithine hydrochloride (O) on heating under reflux provides the six-membered lactam (P) which on further protection with BOC-anhydride produces the desired amide (Q) in reasonable yield. This intermediate can then be carried on to the final product (S) following a method as described in the preceding schemes. For 5-membered lactams, L-ornithine hydrochloride (Compound (O)) is replaced by $(COOH)CH(NH_2)CH_2CH_2NH_2$ as the starting material.

d. General Scheme 4:

Modifications of the ring system described in General Scheme 3 can also be made via the following a method, to provide unsaturation in the Q-containing ring.



Here, a properly-substituted pyrimidone (T) is N-alkylated under appropriate conditions to give (U). The resultant product, in this case the nitro-pyrimidone (U), is reduced to provide the desired amine (V). This intermediate is coupled to the succinate (F) or (G) described in Scheme 1 (see Scheme 1 for the synthesis of final products). A variety of ring systems can be generated in a similar fashion.

Compositions:

The compositions of the invention comprise:

(a) a safe and effective amount of a compound of Formula (I); and

(b) a pharmaceutically-acceptable carrier.

As discussed above, numerous diseases are known to be mediated by excess or undesired matrix-destroying metalloprotease activity. These include tumor metastasis, osteoarthritis, rheumatoid arthritis, skin inflammation, ulcerations, particularly of the cornea, reaction to infection, periodontitis and the like. Thus, the compounds of the invention are useful in therapy with regard to conditions involving this unwanted activity.

The invention compounds can therefore be formulated into pharmaceutical compositions for use in treatment or prophylaxis of these conditions. Standard pharmaceutical formulation techniques are used, such as those disclosed in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., latest edition.

A "safe and effective amount" of a Formula (I) compound is an amount that is effective, to inhibit matrix metalloproteases at the site(s) of activity, in a human or lower animal subject, without undue adverse side effects (such as toxicity, irritation, or allergic response), commensurate with a reasonable benefit/risk ratio when used in the manner of this invention. The specific "safe and effective amount" will, obviously, vary with such factors as the particular condition being treated, the physical condition of the patient, the duration of treatment, the nature of concurrent therapy (if any), the specific dosage form to be used, the carrier employed, the solubility of the Formula (I) compound therein, and the dosage regimen desired for the composition.

The compositions of this invention are preferably provided in unit dosage form. As used herein, a "unit dosage form" is a composition of this invention containing an amount of a Formula (I) compound that is suitable for administration to a human or lower animal subject, in a single dose, according to good medical practice. These compositions preferably contain from about 5 mg (milligrams) to about 1000 mg, more preferably from about 10 mg to about 500 mg, more preferably from about 10 mg to about 300 mg, of a Formula (I) compound.

The compositions of this invention may be in any of a variety of forms, suitable (for example) for oral, rectal, topical or parenteral administration. Depending upon the particular route of administration desired, a variety of pharmaceutically-acceptable carriers well-known in the art may be used. These include solid r liquid fillers, diluents, hydrotropes, surface-active agents, and

encapsulating substances. Optional pharmaceutically-active materials may be included, which do not substantially interfere with the inhibitory activity of the Formula (I) compound. The amount of carrier employed in conjunction with the Formula (I) compound is sufficient to provide a practical quantity of material for administration per unit dose of the Formula (I) compound. Techniques and compositions for making dosage forms useful in the methods of this invention are described in the following references, all incorporated by reference herein: Modern Pharmaceutics, Chapters 9 and 10 (Banker & Rhodes, editors, 1979); Lieberman et al., Pharmaceutical Dosage Forms: Tablets (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms 2d Edition (1976).

In particular, pharmaceutically-acceptable carriers for systemic administration include sugars, starches, cellulose and its derivatives, malt, gelatin, talc, calcium sulfate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffer solutions, emulsifiers, isotonic saline, and pyrogen-free water. Preferred carriers for parenteral administration include propylene glycol, ethyl oleate, pyrrolidone, ethanol, and sesame oil. Preferably, the pharmaceutically-acceptable carrier, in compositions for parenteral administration, comprises at least about 90% by weight of the total composition.

Various oral dosage forms can be used, including such solid forms as tablets, capsules, granules and bulk powders. These oral forms comprise a safe and effective amount, usually at least about 5%, and preferably from about 25% to about 50%, of the Formula (I) compound. Tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, or multiple-compressed, containing suitable binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents, and melting agents. Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules, and effervescent preparations reconstituted from effervescent granules, containing suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, melting agents, coloring agents and flavoring agents.

The compositions of this invention can also be administered topically to a subject, i.e., by the direct laying on or spreading of the composition on the epidermal or epithelial tissue of the subject. Such compositions include, for example, lotions, creams, solutions, gels and solids. These topical compositions preferably comprise a safe and effective amount, usually at least about 0.1%, and preferably from about 1% to about 5%, of the Formula (I) compound. Suitable carriers for topical administration preferably remain in place on the skin as a

continuous film, and resist being removed by perspiration or immersion in water. Generally, the carrier is organic in nature and capable of having dispersed or dissolved therein the Formula (I) compound. The carrier may include pharmaceutically-acceptable emolients, emulsifiers, thickening agents, and solvents.

Methods of Administration:

This invention also provides methods of treating or preventing disorders associated with excess or undesired matrix metalloprotease activity in a human or other animal subject, by administering a safe and effective amount of a Formula (I) compound to said subject. As used herein, a "disorder associated with excess or undesired matrix metalloprotease activity" is any disorder characterized by degradation of matrix proteins. The methods of the invention are useful in treating disorders such as (for example) osteoarthritis, periodontitis, corneal ulceration, tumor invasion, and rheumatoid arthritis.

The Formula (I) compounds and compositions of this invention can be administered topically or systemically. Systemic application includes any method of introducing Formula (I) compound into the tissues of the body, e.g., intra-articular (especially in treatment of rheumatoid arthritis), intrathecal, epidural, intramuscular, transdermal, intravenous, intraperitoneal, subcutaneous, sublingual, rectal, and oral administration. The Formula (I) compounds of the present invention are preferably administered orally.

The specific dosage of inhibitor to be administered, as well as the duration of treatment, are mutually dependent. The dosage and treatment regimen will also depend upon such factors as the specific Formula (I) compound used, the treatment indication, the ability of the Formula (I) compound to reach minimum inhibitory concentrations at the site of the matrix metalloprotease to be inhibited, the personal attributes of the subject (such as weight), compliance with the treatment regimen, and the presence and severity of any side effects of the treatment.

Typically, for a human adult (weighing approximately 70 kilograms), from about 5 mg to about 3000 mg, more preferably from about 5 mg to about 1000 mg, more preferably from about 10 mg to about 100 mg, of Formula (I) compound are administered per day. It is understood that these dosage ranges are by way of example only, and that daily administration can be adjusted depending on the factors listed above.

A preferred method of administration for treatment of rheumatoid arthritis is oral or parenterally via intra-articular injection. As is known and practic d in the art, all formulations for parenteral administration must be sterile. For mammals,

especially humans, (assuming an approximate body weight of 70 kilograms) individual doses of from about 10 mg to about 1000 mg are preferred.

A preferred method of systemic administration is oral. Individual doses of from about 10 mg to about 1000 mg, preferably from about 10 mg to about 300 mg are preferred.

Topical administration can be used to deliver the Formula (I) compound systemically, or to treat a subject locally. The amounts of Formula (I) compound to be topically administered depends upon such factors as skin sensitivity, type and location of the tissue to be treated, the composition and carrier (if any) to be administered, the particular Formula (I) compound to be administered, as well as the particular disorder to be treated and the extent to which systemic (as distinguwashed from local) effects are desired.

The inhibitors of the invention can be targeted to specific locations where the matrix metalloprotease is accumulated by using targeting ligands. For example, to focus the inhibitors to matrix metalloprotease contained in a tumor, the inhibitor is conjugated to an antibody or fragment thereof which is immunoreactive with a tumor marker as is generally understood in the preparation of immunotoxins in general. The targeting ligand can also be a ligand suitable for a receptor which is present on the tumor. Any targeting ligand which specifically reacts with a marker for the intended target tissue can be used. Methods for coupling the invention compound to the targeting ligand are well known and are similar to those described below for coupling to carrier. The conjugates are formulated and administered as described above.

For localized conditions, topical administration is preferred. For example, to treat ulcerated cornea, direct application to the affected eye may employ a formulation as eyedrops or aerosol. For corneal treatment, the compounds of the invention can also be formulated as gels or ointments, or can be incorporated into collagen or a hydrophilic polymer shield. The materials can also be inserted as a contact lens or reservoir or as a subconjunctival formulation. For treatment of skin inflammation, the compound is applied locally and topically, in a gel, paste, salve or ointment. The mode of treatment thus reflects the nature of the condition and suitable formulations for any selected route are available in the art.

In all of the foregoing, of course, the compounds of the invention can be administered alone or as mixtures, and the compositions may further include additional drugs or excipients as appropriate for the indication.

Some of the compounds of the invention also inhibit bacterial metalloproteases although generally at a lower level than that exhibited with respect to mammalian metalloproteases. S me bacterial metalloproteases seem to be less dependent on the

stereochemistry of the inhibitor, whereas substantial differences are found between diastereomers in their ability to inactivate the mammalian proteases. Thus, this pattern of activity can be used to distinguish between the mammalian and bacterial enzymes.

Preparation and Use of Antibodies:

The invention compounds can also be utilized in immunization protocols to obtain antisera immunospecific for the invention compounds. As the invention compounds are relatively small, they are advantageously coupled to antigenically neutral carriers such as the conventionally used keyhole limpet hemocyanin (KLH) or serum albumin carriers. For those invention compounds having a carboxyl functionality, coupling to carrier can be done by methods generally known in the art. For example, the carboxyl residue can be reduced to an aldehyde and coupled to carrier through reaction with sidechain amino groups in protein-based carriers, optionally followed by reduction of imino linkage formed. The carboxyl residue can also be reacted with sidechain amino groups using condensing agents such as dicyclohexyl carbodiimide or other carbodiimide dehydrating agents.

Linker compounds can also be used to effect the coupling; both homobifunctional and heterobifunctional linkers are available from Pierce Chemical Company, Rockford, Ill. The resulting immunogenic complex can then be injected into suitable mammalian subjects such as mice, rabbits, and the like. Suitable protocols involve repeated injection of the immunogen in the presence of adjuvants according to a schedule which boosts production of antibodies in the serum. The titers of the immune serum can readily be measured using immunoassay procedures, now standard in the art, employing the invention compounds as antigens.

The antisera obtained can be used directly or monoclonal antibodies may be obtained by harvesting the peripheral blood lymphocytes or the spleen of the immunized animal and immortalizing the antibody-producing cells, followed by identifying the suitable antibody producers using standard immunoassay techniques.

The polyclonal or monoclonal preparations are then useful in monitoring therapy or prophylaxis regimens involving the compounds of the invention. Suitable samples such as those derived from blood, serum, urine, or saliva can be tested for the presence of the administered inhibitor at various times during the treatment protocol using standard immunoassay techniques which employ the antibody preparations of the invention.

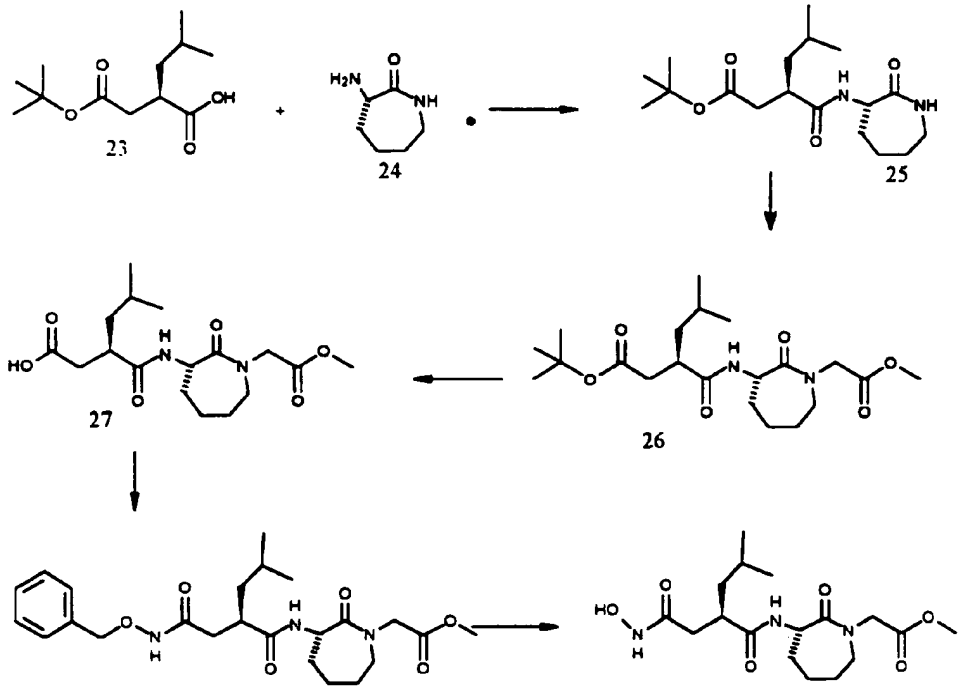
The invention compounds can also be coupled to labels such as scintigraphic labels, e.g., technetium 99 or I-131, using standard coupling methods. The labeled compounds are administered to subjects to determine the locations of excess amounts of

one or more matrix metalloproteases in vivo. The ability of the inhibitors to selectively bind matrix metalloprotease is thus taken advantage of to map the distribution of these enzymes in situ. The techniques can also be employed in histological procedures and the labeled invention compounds can be used in competitive immunoassays.

The following non-limiting examples illustrate the compounds, compositions, and uses of the present invention.

Example 1

Synthesis of (2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propanoic acid amide of 1*N*-(carbomethoxymethyl)-caprolactam-(3S)-amine (1)



(2R)-Isobutyl-3-carbo-*tert*-butoxypropionic acid amide of caprolactam-(3S)-amine (25). A mixture of acid **23** (2.0 g, 8.70 mmole), caprolactam-[3S]-amine **24** (1.23 g, 9.57 mmole) and 1-Hydroxybenzotriazole hydrate ("HOBT") (4.0 g, 26.1 mmole) in 40 mL of DMF and 1.6 mL of *N*-Methylmorpholine ("NMM") is charged with 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide ("EDAC") (2.0 g, 10.44 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and ethyl acetate ("EtOAc"). The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, evaporated and chromatographed over flash silica with EtOAc to give **25**.

(2R)-Isobutyl-3-carbo-*tert*-butoxypropanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine (**26**). The caprolactam **25** (914 mg, 2.69 mmole) is taken in 10 mL THF and cooled to -78°C under argon. To this is added 1 M lithium bis(trimethylsilyl)amide (2.69 mL, 2.69 mmole) and the reaction stirred for 5 min. Methyl bromoacetate (256 µL, 2.69 mmole) is added and stirred for 2 hr. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give crude material which is then chromatographed over flash silica with hexanes:EtOAc (1:4) to give **26**.

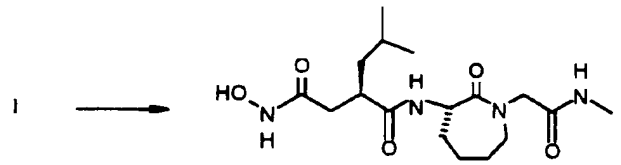
(2R)-Isobutyl-3-carboxypropanoic acid amide of N-(carbomethoxymethyl)-caprolactam-(3S)-amine (**27**). Trifluoroacetic acid (3 mL) is added via syringe to a solution of *tert*-butyl ester **26** (380 mg, 0.922 mmole) in 3 mL CH_2Cl_2 under argon and the resulting mixture is stirred for 2 hr at room temperature. The material is then concentrated under vacuum to give **27** which is carried forward without purification.

(2R)-Isobutyl-3-(*O*-benzyl-*N*-hydroxycarboxamido)-propanoic acid amide of 1*N*-(carbomethoxymethyl)-caprolactam-(3S)-amine (**28**). A mixture of acid **27** (345 mg, 0.863 mmole), *O*-benzyl hydroxylamine hydrochloride (166 mg, 1.035 mmole) and HOBT (397 mg, 2.59 mmole) in 5 mL of *N,N*-Dimethylformamide ("DMF") and 260 µL of NMM is charged with EDAC (199 mg, 1.035 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give crude material which is then chromatographed over flash silica with EtOAc to give the title compound **28**.

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine (**1**). The benzyl hydroxamic acid **28** (325 mg, 0.705 mmole) is taken in 6 mL EtOH and the mixture is charged with 10% palladium on carbon (60 mg) and stirred under one atmosphere of hydrogen for 45 min. The mixture is then filtered through celite and concentrated to give 240 mg of crude material which is then chromatographed over flash silica with EtOAc:formic acid (98:2) and then recrystallized from hexanes:EtOAc (2:1) to give the pure desired hydroxamic acid **1**.

Example 2

Synthesis of (2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propionic acid amide of 1*N*-(methyl-carboxamidomethyl)-caprolactam-(3S)-amine (2)



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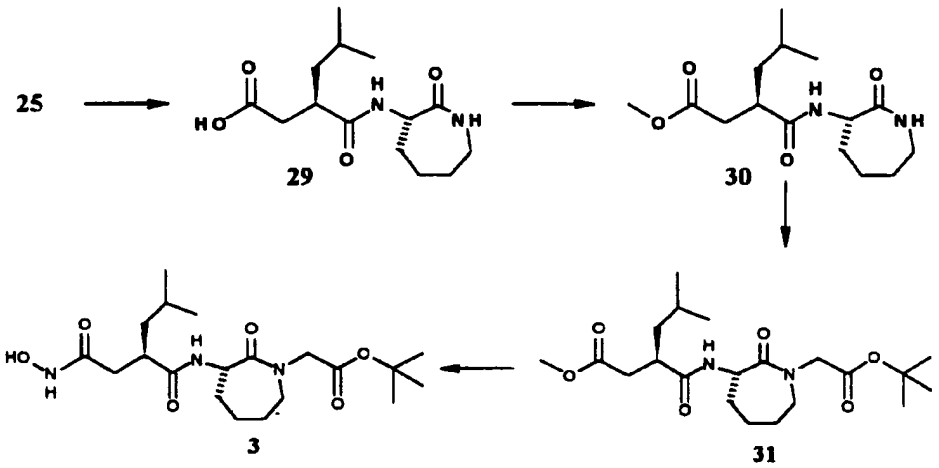
(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propionic acid amide of 1*N*-(methyl-carboxamidomethyl)-caprolactam-(3S)-amine (2). The methyl ester **1** (80 mg, .216 mmole) is taken in 5 mL of 8M methyl amine in MeOH and stirred for 15 hours. The solvent is removed and the residue • chromatographed over flash silica with EtOAc:formic acid (97:3) to give **2**.

Example 3

Synthesis of (2R)-Isobutyl-3-(N-hydroxycarboxamido)-propionic acid amide of 1N-(carbo-tert-butoxy-methyl)-caprolactam-(3S)-amine (3)



(2R)-Isobutyl-3-carboxypropionic acid amide of caprolactam-(3S)-amine (29). Trifluoroacetic acid (15 mL) is added via syringe to a solution of *tert*-butyl ester 25 (2.2 g, 6.47 mmole) in 15 mL CH_2Cl_2 under argon and the resulting mixture is stirred for 2 hr at room temperature. The material is then concentrated under vacuum to give **29** which is carried forward without purification.

(2R)-Isobutyl-3-carbomethoxypropionic acid amide of caprolactam-(3S)-amine (30). To a solution of acid **29** (1.24 g, 4.37 mmole) in 5 mL MeOH is added an excess of diazomethane in ether. The excess diazomethane is then quenched with acetic acid

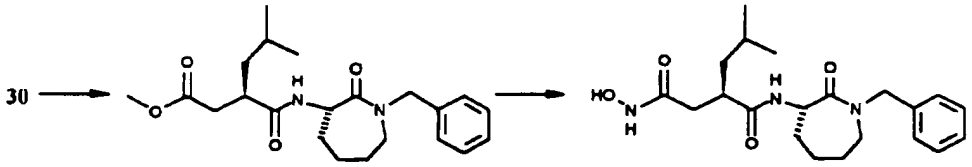
and the solvent is evaporated. The residue is chromatographed over flash silica with EtOAc to give the desired ester **30**.

(2R)-Isobutyl-3-carbomethoxypropionic acid amide of 1*N*-(carbo-*tert*-butoxy-methyl)-caprolactam-(3S)-amine (**31**). The caprolactam **30** (200 mg, 0.67 mmole) is taken in 5 mL dry tetrahydrofuran ("THF") and cooled to -78°C under argon. To this solution is added 1 M lithium bis(trimethylsilyl)amide (0.67 mL, 0.67 mmole) and the reaction is stirred for 5 min. *tert*-Butyl bromoacetate (99 µL, 0.67 mmole) is added and stirred for 2 hr. The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give a crude material which is chromatographed over flash silica with hexanes:EtOAc (1:4) to give pure desired ester **31**.

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propionic acid amide of 1*N*-(carbo-*tert*-butoxy-methyl)-caprolactam-(3S)-amine (**3**). Ester **31** (130 mg, 0.29 mmole) is added to NH_2OK (1.3 mL, 1 eq in MeOH, prepared according to Fieser and Fieser, Reagents for Organic Synthesis, Vol. 1, p. 478 (1967)) and stirred for 24 hr. The solvent is evaporated and the residue is dissolved in 1N HCl and extracted with EtOAc. The organic layer is dried over $MgSO_4$, evaporated and the residue is chromatographed over flash silica with EtOAc:formic acid (98:2) to give **3**.

Example 4

Synthesis of (2R)-Isobutyl-3-(N-hydroxycarboxamido)-propionic acid amide of 1N-Benzyl-caprolactam-(3S)-amine (**4**).



32 **4**

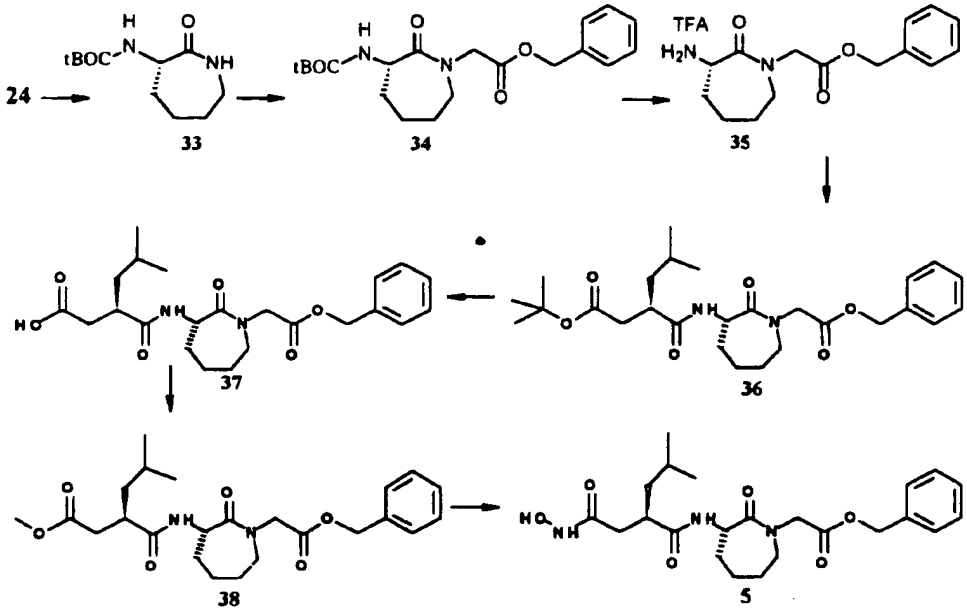
(2R)-Isobutyl-3-carbomethoxypropionic acid amide of 1*N*-Benzylcaprolactam-(3S)-amine (**32**). The caprolactam **30** (200 mg, 0.67 mmole) is taken in 5 mL dry THF and is cooled to -78°C under argon. To this is added 1 M lithium bis(trimethylsilyl)amide (0.67 mL, 0.67 mmole) and the reaction stirred for 5 min. Benzyl bromide (80 µL, 0.67 mmole) is added and stirred for 2 hr The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give a crude oil which is chromatographed over flash silica with hexanes:EtOAc (1:4) to give **32**.

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propionic acid amide of 1*N*-Benzyl-caprolactam-(3S)-amine (**4**). Ester **32** (200 mg, 0.52 mmole) is added to is added to NH_2OK (1.0 mL, 1 eq in MeOH, prepared according to Fieser and Fieser, Vol 1, p 478)

and stirred for 24 hr. The solvent is evaporated and the residue is dissolved in 1N HCl and extracted with EtOAc. The organic layer is dried over $MgSO_4$, evaporated, and the residue is chromatographed over flash silica with EtOAc:formic acid (98:2) to give **4**.

Example 5

Synthesis of(2R)-Isobutyl-3-N-hydroxycarboxamidopropionic acid amide of 1N-(carbobenzyloxy-methyl)-caprolactam-(3S)-amine (5)



tert-Butoxycarboxylic acid amide of caprolactam-(3S)-amine (**33**). A solution of **24** (49.0 g, 383 mmole) in 350 mL DMSO is charged with di-*tert*-butyl dicarbonate (83.5 g, 383 mmole) and stirred for 5 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, and brine, dried over $MgSO_4$, filtered and evaporated. The residue is recrystallized from ether:hexanes (2:1) to give **33**.

tert-Butoxycarboxylic acid amide of 1*N*-(carbobenzyloxymethyl)-caprolactam-(3S)-amine (**34**). Lithium bis(trimethylsilyl)amide (44.7 mL, 44.7, 1 M in THF) is added to a solution of the caprolactam **33** (10.2 g, 44.7 mmol) in THF (100 mL) at -78° C under argon and stirred for 15 min. Benzyl bromoacetate (7.08 mL, 44.7 mmol) is added to the solution via syringe, warmed to room temperature, and stirred for 2 hr. The reaction is partitioned between H_2O and EtOAc. The organic layer is washed with aqueous $NaHCO_3$, aqueous NaCl, and dried over $MgSO_4$. The crude product is chromatographed on flash silica with hexane:EtOAc (1:1) to give **34**.

1*N*-(carbobenzyloxymethyl)-caprolactam-(3S)-amine trifluoroacetic acid salt (**35**). Trifluoroacetic acid (15 mL) is added via syringe to a solution of *tert*-butyl

carboxamate **34** (5.0 g, 13.2 mm le) in 15 mL under argon and the resulting mixture is stirred for 1 hr at room temperature. The material is then concentrated under vacuum to give **35** which is carried forward without purification.

(2R)-Isobutyl-3-carbo-*tert*-butoxypropionic acid amide of 1*N*-(carbobenzyloxy-methyl)-caprolactam-(3S)-amine (**36**). A mixture of acid **23** (1.67, 7.25 mmole), caprolactam **35** (2.0 g, 7.25 mmole) and HOBT (2.94 g, 21.75 mmole) in 15 mL of DMF and 1.5 mL of NMM is charged with EDAC (1.67 g, 8.70 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc and then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, evaporated and chromatographed over flash silica with EtOAc:hexanes (2:1) to give pure desired amide **36**.

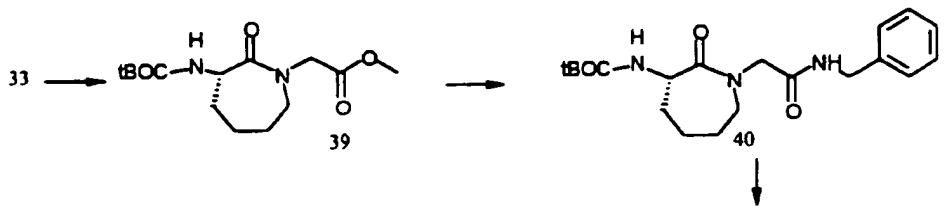
(2R)-Isobutyl-3-carbomethoxypropionic acid amide of 1*N*-(carbobenzyloxy-methyl)-caprolactam-(3S)-amine (**38**). Trifluoroacetic acid (5 mL) is added via syringe to a solution of *tert*-butyl ester **36** (1.2 g, 2.46 mmole) in 5 mL of CH_2Cl_2 under argon and the resulting mixture is stirred for 2 hr at room temperature. The material is then concentrated under vacuum to give acid **37** as a clear oil which is carried forward without purification.

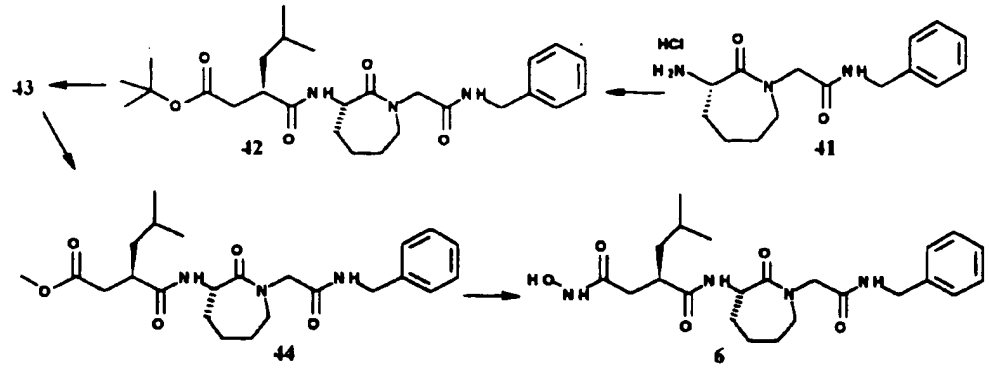
To a solution of acid **37** (1.06 g, 2.46 mmole) in 5 mL MeOH is added an excess of diazomethane in ether. The excess diazomethane is then quenched with acetic acid and the solvent evaporated. The residue is chromatographed over flash silica with EtOAc:hexanes (2:1) to give the desired ester **38**.

(2R)-Isobutyl-3-*N*-hydroxycarboxamidopropionic acid amide of 1*N*-(carbo-benzyloxy-methyl)-caprolactam-(3S)-amine (**5**). Ester **38** (420 mg, 0.94 mmole) is added to a solution of NH_2OK (1.3 mL, 1 eq in MeOH, prepared according to Fieser and Fieser, Vol 1, p 478) and stirred for 24 hours. The solvent is then evaporated and the residue dissolved in 1N HCl and extracted with EtOAc. The organic layer is dried over $MgSO_4$, evaporated and the residue chromatographed over flash silica with EtOAc:formic acid (98:2) to give **5**.

Example 6

Synthesis of (2R)-Isobutyl-3-(N-hydroxycarboxamido)-propionic acid amide of 1N-(N-Benzyl-carboxamidomethyl)-caprolactam-(3S)-amine (**6**)





tert-Butoxycarboxylic acid amide of 1*N*-(carbomethoxymethyl)-caprolactam-(3S)-amine (39). Lithium bis(trimethylsilyl)amide (83.3 mL, 83.3, 1 M in THF) is added to a solution of the caprolactam **33** (19.0 g, 83.3 mmol) in THF (200 mL) at -78° C under argon and stirred for 15 min. Methyl bromoacetate (7.88 mL, 83.3 mmol) is added to the solution via syringe, warmed to room temperature, and stirred for 1 hr. The reaction is partitioned between H_2O and EtOAc. The organic layer is washed with aqueous $NaHCO_3$, aqueous NaCl, and dried over $MgSO_4$. The crude product is chromatographed on flash silica with EtOAc to give **39**.

tert-Butoxycarboxylic acid amide of 1*N*-(*N*-benzylcarboxamidomethyl)-caprolactam-(3S)-amine (40). The methyl ester **39** (2.5 g, 8.33 mmole) is taken in 10 mL MeOH and the mixture is charged with benzyl amine (8.7 mL, 79.7 mmole) and is stirred for 15 hours. The solvent is removed and the residue is chromatographed over flash silica with EtOAc:hexane (1:1) to give **40**.

1*N*-(*N*-Benzylcarboxamidomethyl)-caprolactam-(3S)-amine hydrochloride (41). The amide **40** (2.2 g, 5.87 mmole) is taken in 50 mL ether at 0° C and dry HCl is bubbled through for 10 min. The solid is filtered and washed with ether to give **41**.

(2R)-Isobutyl-3-carbo-*tert*-butoxypropionic acid amide of 1*N*-(*N*-benzylcarboxamido-methyl)-caprolactam-(3S)-amine (42). A mixture of acid **23** (1.0 g, 4.35 mmole), caprolactam **41** (1.35 g, 4.35 mmole) and HOBT (2.0 g, 13.0 mmole) in 15 mL of DMF and 1.3 mL of NMM is charged with EDAC (1.0 g, 5.22 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give 1.9 g of crude solid which is chromatographed over flash silica with EtOAc to give **42**.

(2R)-Isobutyl-3-carboxypropionic acid amide of 1*N*-(*N*-benzylcarbox-amidomethyl)-caprolactam-(3S)-amine (43). Trifluoroacetic acid (5 mL) is added via syringe to a solution of *tert*-butyl ester **42** (1.51 g, 3.10 mmole) in 5 mL CH_2Cl_2 under argon and the resulting mixture is stirred for 2 hr at room temperature. The material is

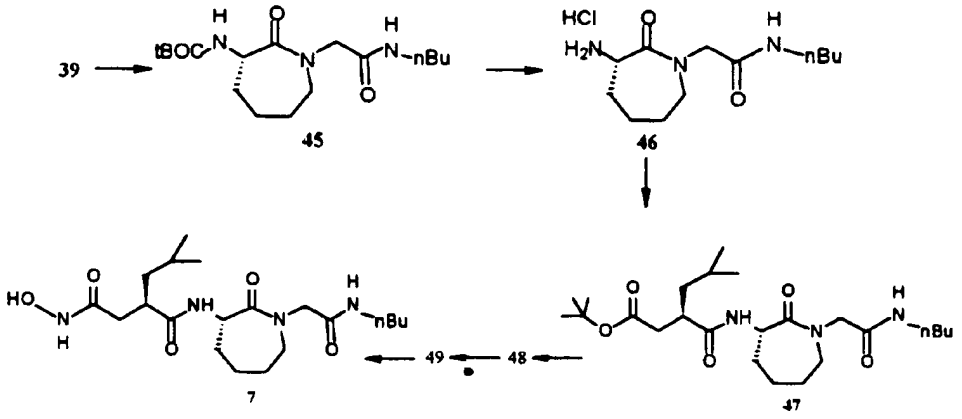
then concentrated under vacuum to give **43** which is carried forward without purification.

(2R)-Isobutyl-3-carbomethoxypropionic acid amide of 1*N*-(*N*-benzylcarboxamido-methyl)-caprolactam-(3S)-amine (44). To a solution of acid **43** (1.51 g, 3.50 mmole) in 5 mL MeOH is added an excess of diazomethane in ether. The excess diazomethane is quenched with acetic acid and the solvent evaporated. The residue is chromatographed over flash silica with EtOAc:hexanes (2:1) to give **44**.

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propionic acid amide of 1*N*-(*N*-benzylcarboxamidomethyl)-caprolactam-(3S)-amine (6). Ester **44** (800 mg, 1.80 mmole) is added to a solution of NH_2OK (1.5 mL, 1 eq in MeOH, prepared according to Fieser and Fieser, Vol 1, p 478) and stirred for 24 hours. The solvent is evaporated and the residue is dissolved in 1N HCl and extracted with EtOAc. The organic layer is dried over $MgSO_4$, filtered, evaporated and the residue is chromatographed over flash silica with EtOAc:formic acid (97:3) to give **6**.

Example 7

Synthesis of(2R)-Isobutyl-3-*N*-hydroxycarboxamidopropionic acid amide of 1*N*-(*n*-butylcarboxamidomethyl)-caprolactam-(3S)-amine (7)



tert-Butoxycarboxylic acid amide of 1*N*-(*n*-butylcarboxamidomethyl)-caprolactam-(3S)-amine (45). The methyl ester **39** (2.5 g, 8.33 mmole) is taken in 10 mL MeOH and the mixture is charged with butyl amine (8.1 mL, 79.7 mmole) and stirred for 15 hr. The solvent is removed and the residue is chromatographed over flash silica with EtOAc:hexane (2:1) to give **45**.

1*N*-(*n*-butylcarboxamidomethyl)-(3S)-aminocaprolactam hydrochloride (46). The amide **45** (2.2 g, 6.43 mmole) is taken in 50 mL ether at 0° C and dry HCl is bubbled through for 10 min. The solid is filtered and washed with ether to give **46**.

(2R)-Isobutyl-(3)-carbo-*tert*-butoxypropanoic acid amide of (1*N*)-*n*-butylcarboxamido-methyl-caprolactam-(3S)-amine (47). A mixture of acid **23**, (1.76 g, 7.65 mmole), 1*N*-(*n*-butylcarboxamidomethyl)-caprolactam-(3S)-amine **46** (3.17 g, 11.4 mmole) and HOBT (3.08g. 22.80 mmole) in 20 mL of DMF and 2.36 mL of NMM is charged with EDAC (1.74 g, 9.07 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give 4.68 g of crude solid which is chromatographed over flash silica with EtOAc to give **47**.

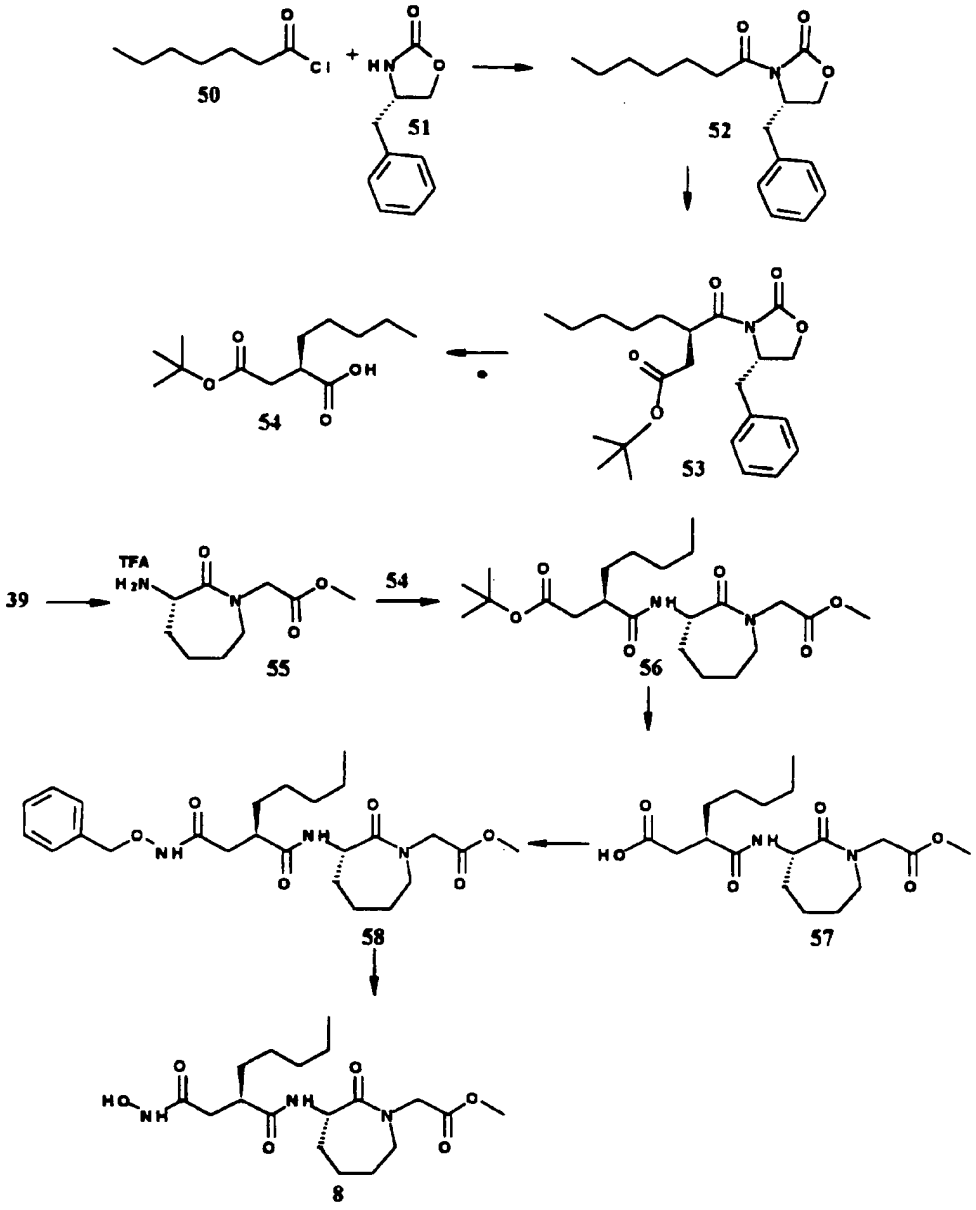
(2R)-Isobutyl-(3)-carboxypropanoic acid amide of (1*N*)-*n*-butylcarbox-amidomethyl-caprolactam-(3S)-amine (48). Trifluoroacetic acid (15 mL) is added via syringe to a solution of the *tert*-butyl ester **47** (2.45 g, 5.40 mmole) in 15 mL of CH_2Cl_2 under argon and the resulting mixture is stirred for 1 hr at room temperature. The material is then concentrated under vacuum to give **48** which is carried forward without purification.

(2R)-Isobutyl-3-carbomethoxypropionic acid amide of 1*N*-(*n*-butylcarboxamidomethyl)-caprolactam-(3S)-amine (49). The acid **48** (1.23 g, 3.09 mmole) is taken in methanol and treated with an excess of diazomethane in ether at 0°C, and stirred for one hour. Formic acid (3 drops) is added and the mixture is evaporated to dryness to give **49** which is carried forward without purification.

(2R)-Isobutyl-3-*N*-hydroxycarboxamidopropionic acid amide of 1*N*-(*n*-butylcarboxamidomethyl)-caprolactam-(3S)-amine (7). The methyl ester **49** (500 mg, 1.21 mmole) is added to a solution of NH_2OK (1.4 mL, 1 eq in MeOH, prepared according to Fieser and Fieser, Vol 1, p 478) and stirred for 15 hr. The reaction mixture is acidified with acetic acid to pH = 2. The reaction is then partitioned between ethyl acetate and water. The organic layer is then washed with brine, and dried over magnesium sulfate, filtered and evaporated to give a crude solid which is chromatographed with ethyl acetate:water:acetic acid (16:1:1) and then recrystallized from ethyl acetate to give **7**.

Example 8

Synthesis of(2R)-N-Hydroxycarboxamidomethyl heptanoic acid amide of N-(carbomethoxymethyl)-caprolactam-(3S)-amine (8)



3-(1-Oxoheptyl)-(4S)-phenylmethyl-2-oxazolidinone (52). *n*-Butyl lithium (58 mL, 2.5 M in hexanes, 142 mmol) is added to a solution of (S)-4-benzyl-2-oxazolidinone 51 (25 g, 141 mmol) in THF (250 mL) at -78° C under argon and stirred for 15 min. Heptanoyl chloride **50** (21 g, 141 mmol) is added to the solution dropwise

and stirred for 40 min, then warmed to 0° C for 2 hours. The reaction is quenched with NH_4Cl and extracted with EtOAc. The organic layer is washed with 1 N HCl, aqueous $NaHCO_3$, aqueous NaCl, and dried over $MgSO_4$. The crude product is recrystallized from hexane to give **52**.

3-[1-Oxo-(2R)-(carbo-*tert*-butoxymethyl)-heptyl]-(4S)-phenylmethyl-2-oxazolidinone (53). Lithium bis(trimethylsilyl)amide (132 mL, 132 mmol, 1 M in THF) is added to a solution of the oxazolidinone **52** (38.0 g, 132 mmol) in THF (100 mL) at -78° C under argon and stirred for 15 min. *tert*-Butyl bromoacetate (26 mL, 132 mmol) is added to the solution via syringe and stirred for 3 hr. The reaction is then warmed to 0° C and stirred for 1.5 hr. The reaction is quenched with NH_4Cl and extracted with EtOAc. The organic layer is washed with 1 N HCl, aqueous $NaHCO_3$, aqueous NaCl, and dried over $MgSO_4$. The crude product is recrystallized from hexane:EtOAc (2:1) to give **53**.

tert-Butyl-[(3R)-carboxyloctanoate (54). The oxazolidinone 53 (10.0 g, 24.9 mmole) is dissolved in THF/H_2O (100 mL : 25 mL) under argon and cooled to 0° C. Hydrogen peroxide (12 mL, 30%, 106.7 mmol) is added dropwise to the solution, followed by lithium hydroxide monohydrate (1.8 g, 43.9 mmol) in H_2O (40 mL). The reaction is stirred for 3 hr, at which time sodium sulfite (10 g in 40 mL H_2O) is added dropwise and stirred for 20 min. The solution is extracted 3 times with CH_2Cl_2. The organic extracts are combined, washed with aqueous NaCl, dried over $MgSO_4$, filtered, and the solvent removed to give **54**.

1*N*-(carbomethoxymethyl)-caprolactam-(3S)-amine trifluoroacetic acid salt (55). Trifluoroacetic acid (15 mL) is added via syringe to a solution of *tert*-butyl carboxamate **39** (10.0 g, 33.3 mmole) in 15 mL under argon and the resulting mixture is stirred for 1 hr at room temperature. The material is then concentrated under vacuum to give **55** which is carried forward without purification.

(2R)-Carbo-*tert*-butoxymethyl heptanoic acid amide of *N*-carbomethoxymethyl-caprolactam-(3S)-amine (56). The amine **55** (2.16 g, 10.8 mmole) is mixed with the acid **54** (2.64 g, 10.8 mmole), HOBT (4.4 g, 32.4 mmole), EDAC (2.69 g, 14 mmole) in 15 mL of DMF and 2.4 mL (21.6 mmole) of NMM at 0°C, and stirred for 17 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, H_2O, 1N NaOH, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil (3.2 g) which is chromatographed over flash silica with hexane:EtOAc (1:1) to give **56**.

(2R)-*O*-Benzyl-*N*-hydroxycarboxamidomethyl heptanoic acid amide of *N*-carbomethoxymethyl-caprolactam-(3S)-amine (58). The ester **56** (1.2 g, 2.64 mmole) is dissolved in 15 mL of methylene chloride and cooled to 0°C and trifluoroacetic acid

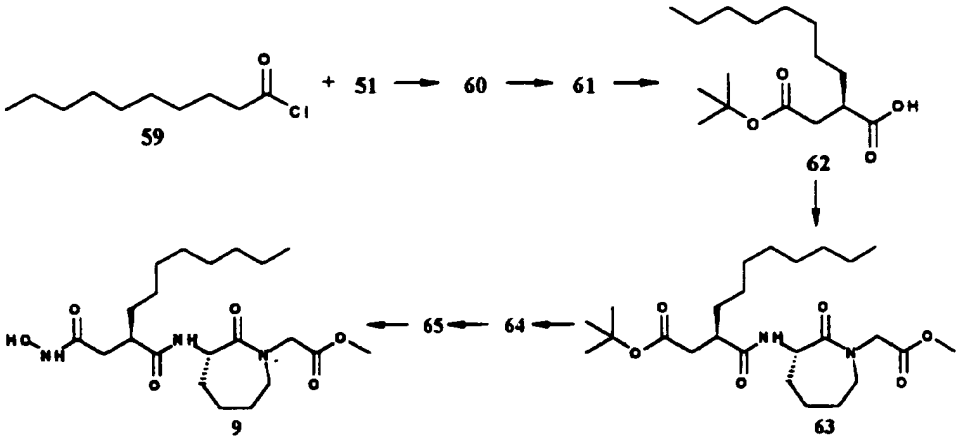
(15 mL) is added slowly. The mixture is stirred for 1.5 hr at room temperature and evaporated to **57**.

A mixture of the crude acid **57** (1.5 g, 2.64 mmole), *O*-benzyl hydroxylamine hydrochloride (0.51 g, 3.17 mmole) and HOBT (1.07 g, 7.92 mmole) in 10 mL of DMF and 0.99 mL of NMM (9.0 mmole) is charged with EDAC (0.61 g, 3.17 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with EtOAc to give **58**.

(2R)-*N*-Hydroxycarboxamidomethyl heptanoic acid amide of *N*-(carbomethoxymethyl)-caprolactam-(3S)-amine (**8**). The benzyl hydroxamic acid **58** (370 mg, 0.78 mmole) is taken in 10 mL of EtOH and the mixture is charged with 10% palladium on carbon (44 mg) and stirred under one atmosphere of hydrogen for 3 hr. The mixture is then filtered through celite and concentrated to an oil. The crude product is crystallized from ethyl acetate to give **8**.

Example 9

Synthesis of(2R)-N-hydroxycarboxamidemethyldecanoic acid amide of 1N-(carbomethoxymethyl)-caprolactam-(3S)-amine (**9**)



3-(1-Oxodecyl)-(4S)-phenylmethyl-2-oxazolidinone (**60**). *n*-Butyl lithium (160 mL, 398 mmol, 2.5 M in hexanes) is added to a solution of (S)-4-benzyl-2-oxazolidinone 51 (64 g, 362 mmol) in THF (750 mL) at -78° C under argon and stirred for 15 min. Decanoyl chloride **59** (69 g, 362 mmol) is added to the solution dropwise and stirred for 40 min, then warmed to 0° C for 2 hours. The reaction is quenched with NH_4Cl and extracted with EtOAc. The organic layer is washed with 1 N HCl, aqueous $NaHCO_3$, aqueous NaCl, and dried over $MgSO_4$. The crude product is recrystallized from hexane to give **60**.

3-[1-Oxo-(2R)-(carbo-*tert*-butoxymethyl)-decyl]-(4S)-phenylmethyl-2-oxazolidinone (61). Lithium bis(trimethylsilyl)amide (210 mL, 1 M in THF, 210 mmol) is added to a solution of the oxazolidinone **60** (66.6 g, 200 mmol) in THF (100 mL) at -78° C under argon and stirred for 15 min. *tert*-Butyl bromoacetate (31 mL, 200 mmol) is added to the solution via syringe and stirred for 3 hr. The reaction is warmed to 0° C and stirred for 1.5 hr. The reaction is quenched with NH_4Cl and extracted with EtOAc. The organic layer is washed with 1 N HCl, aqueous $NaHCO_3$, aqueous NaCl, and dried over $MgSO_4$. The crude product is chromatographed on flash silica with hexane:EtOAc (7:1) to give **61**.

tert-Butyl-[3R-carboxy]undecanoate (62). Oxazolidinone **61** is dissolved in THF/H_2O (100 mL:25 mL) under argon and cooled to 0° C. Hydrogen peroxide (13 mL, 30%, 115.6 mmol) is added dropwise to the solution, followed by lithium hydroxide monohydrate (1.8 g, 43.9 mmol) in H_2O (40 mL). The reaction is stirred for 3 hr, at which time sodium sulfite (10 g in 40 mL H_2O) is added dropwise and stirred 20 min. The solution is extracted 3 times with CH_2Cl_2. The organic extracts are combined, washed with aqueous NaCl, and dried over $MgSO_4$. The product is purified on a silica gel column using EtOAc as the eluent to give **62**.

(2R)-Carbo-*tert*-butoxymethyldecanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine (63). A mixture of acid **62** (2.5 g, 8.74 mmole) and 0.9 mL NMM is cooled to -20°C and charged with isobutyl chloroformate (1.45 mL, 8.74 mmole) and stirred for 10 min. The amine **55** (1.75 g, 8.74 mmole) and 0.9 mL NMM in 2 mL DMF is added and stirred for 30 min. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give 2.8 g of crude solid which chromatographed over flash silica with EtOAc:hexanes (1:1) to give **63**.

(2R)-*O*-Benzyl-*N*-hydroxycarboxamidemethyldecanoic acid amide of 1*N*-(carbomethoxymethyl)-caprolactam-(3S)-amine (65). Trifluoroacetic acid (5 mL) is added via syringe to a solution of *tert*-butyl ester **63** (1.2 g, 2.56 mmole) in 5 mL CH_2Cl_2 under argon and the resulting mixture is stirred for 2 hr at room temperature. The material is then concentrated under vacuum to give **64** which is carried forward without purification.

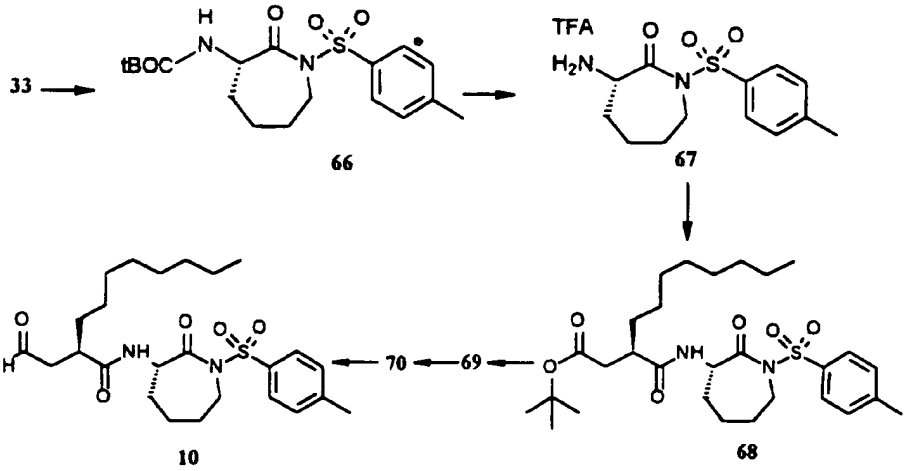
A mixture of acid **64** (1.05 g, 2.55 mmole), *O*-benzyl hydroxylamine hydrochloride (165 mg, 2.55 mmole) and HOBT (1.2 g, 7.65 mmole) in 15 mL of DMF and 6 mL of NMM is charged with EDAC (572 mg, 2.55 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried

over $MgSO_4$, filtered, evaporated and chromatographed over flash silica with EtOAc to give **65**.

(2R)-*N*-hydroxycarboxamidemethyldecanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine (9). The benzyl hydroxamic acid **65** (428 mg, .828 mmole) is taken in 15 mL EtOH and the mixture is charged with 10% palladium on carbon (60 mg) and stirred under one atmosphere of hydrogen for 45 min. The mixture is then filtered through celite and concentrated and chromatographed over flash silica with EtOAc:formic acid (99:1) and then recrystallized from EtOAc to give **9**.

Example 10

Synthesis of (2R)-N-Hydroxycarboxamidomethyl decanoic acid amide of N-p-toluenesulfonyl-caprolactam-(3S)-amine (10)



(3S)-*tert*-Butoxycarbonylamino-*N*-toluenesulfonyl-caprolactam (66). (3S)-*tert*-Butoxy-carbonylamino caprolactam **33** (2.0 g, 8.8 mmole) is dissolved in THF (20 mL) and cooled to 0°C. A solution of lithium bis(trimethylsilyl)amide (10.6 mL, 10.6 mmole, 1M in THF) is added dropwise. After 15 min, toluenesulfonyl chloride (2.0 g, 10.56 mmole) is added. The resulting mixture is stirred at 0°C for 10 min and room temperature for 30 min. The reaction is quenched by water and extracted by ethyl acetate. The organic layer is then washed with 0.1N HCl, H_2O, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with hexanes:EtOAc (3:1) to give **66**.

(2R)-Carbo-*tert*-butoxymethyl decanoic acid amide of *N*-toluenesulfonyl-caprolactam-(3S)-amine (68). (3S)-*tert*-Butoxycarbonylamino-*N*-toluenesulfonyl-caprolactam **66** (2.2 g, 5.8 mmole) is dissolved in 10 mL of methylene chloride and trifluoroacetic acid (10 mL) is added slowly. The mixture is stirred for 30 min. and evaporated to give **67**.

The caprolactam amine **67** (1.6 g, 5.7 mmole) is mixed with **62**, HOBT (2.31 g. 17.1 mmole), EDAC (1.34 g, 7 mmole) in 25 mL of DMF and 1.26 mL (11.4 mmole) of NMM at 0°C, and stirred for 17 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil (3.1 g) which is chromatographed over flash silica with EtOAc to give **68**.

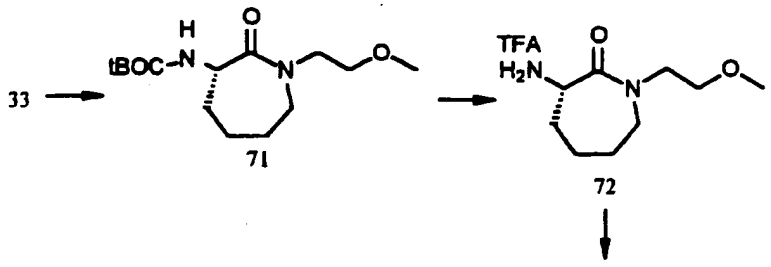
(2R)-*O*-Benzyl-*N*-hydroxycarboxamidomethyl decanoic acid amide of *N*-toluenesulfonyl-caprolactam-(3S)-amine (70). (3S)-*tert*-Butoxycarbonylamino-*N*-toluenesulfonyl-caprolactam **68** (2.1 g, 3.8 mmole) is dissolved in 10 mL of methylene chloride, cooled to 0° C, and trifluoroacetic acid (10 mL) is added slowly. The mixture is stirred for 1.5 hr at room temperature and evaporated to give **69**.

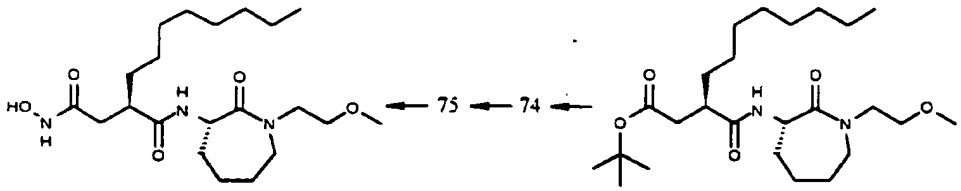
A mixture of the crude acid **69** (2 g, 3.8 mmole), *O*-benzyl hydroxylamine hydrochloride (0.73 g, 4.56 mmole) and HOBT (1.54 g. 11.4 mmole) in 20 mL of DMF and 1.4 mL of NMM (12.9 mmole) is charged with EDAC (0.87g, 4.56 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, 1N NaOH, and brine, dried over $MgSO_4$, filtered and evaporated to give a semisolid (2.2 g) which is chromatographed over flash silica with EtOAc:hexane (1:1) to give **70**.

(2R)-*N*-Hydroxycarboxamidomethyl decanoic acid amide of *N*-toluenesulfonyl-caprolactam-(3S)-amine (10). The benzyl hydroxamic acid **70** (680 mg, 1.13 mmole) is taken in 10 mL of EtOH and the mixture is charged with 10% palladium on carbon (68 mg) and stirred under one atmosphere of hydrogen for 2.5 hr. The mixture is filtered through celite and concentrated to give a solid, which is purified by flash chromatography (silica, EtOAc) to give **10**.

Example 11

Synthesis of 2R)-N-Hydroxycarboxamidomethyl decanoic acid amide of N-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (11)





11 73

(3S)-*tert*-Butoxycarbonylamino-*N*-[(2-methoxy)ethyl]-caprolactam (71). (3S)-*tert*-Butoxycarbonylamino caprolactam **33** (2.0 g, 8.8 mmole) is dissolved in DMF (10 mL). Potassium *tert*-butoxide (1.25 g, 10.6 mmole) is added dropwise, and stirred for 45 min. Bromo ethyl methyl ether (0.99 mL, 10.6 mmole) is then added. The resulting mixture is stirred for 3 hr, at which time the reaction is quenched by water and extracted with ethyl acetate. The organic layer is then washed with 1N HCl, H_2O, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with hexanes:EtOAc (1:1) to give **71**.

(2R)-Carbo-*tert*-butoxymethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (73). (3S)-*tert*-Butoxycarbonylamino-N-[(2-methoxy)ethyl]-caprolactam **71** (1.46 g, 5.1 mmole) is dissolved in 15 mL of methylene chloride, cooled to 0°C, and trifluoroacetic acid (15 mL) is added slowly. The mixture is stirred for 2 hr at room temperature and evaporated to **72**.

The caprolactam amine **72** is mixed with (2R)-*tert*-Butylcarboxymethyl decanoic acid (1.5 g, 5.2 mmole), HOBT (2.07 g, 15.3 mmole), EDAC (1.17 g, 6.1 mmole) in 15 mL of DMF and 1.43 mL (13 mmole) of NMM at 0°C, and stirred for 17 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, H_2O, 1N NaOH, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with EtOAc to give **73**.

(2R)-*O*-Benzyl-*N*-hydroxycarboxamidomethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (75). (3S)-*tert*-Butoxycarbonylamino-*N*-[(2-methoxy)ethyl]-caprolactam **73** (1.2 g, 2.64 mmole) is dissolved in 15 mL of methylene chloride, cooled to 0°C, and trifluoroacetic acid (15 mL) is added slowly. The mixture is stirred for 1.5 hr at room temperature and evaporated to give **74**.

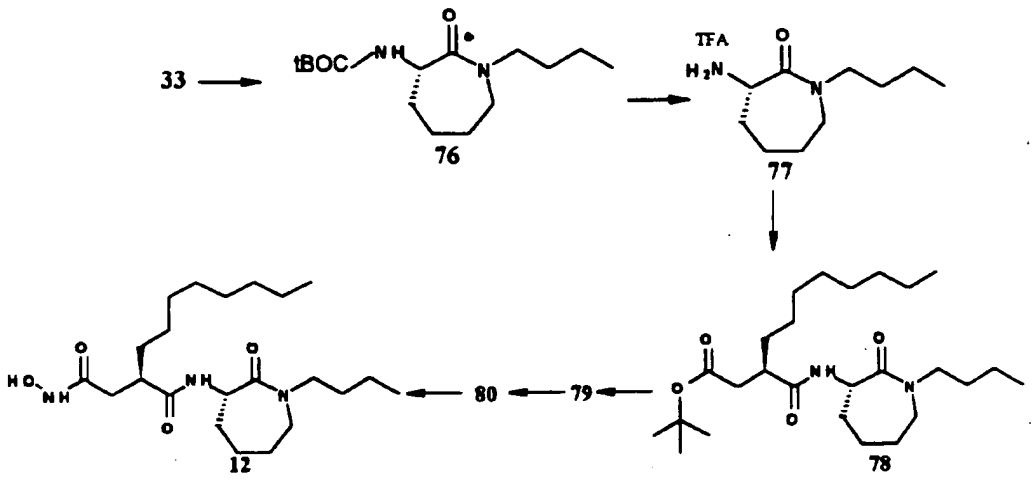
A mixture of the crude acid **74** (1.5 g, 2.64 mmole), *O*-benzyl hydroxylamine hydrochloride (0.51 g, 3.17 mmole) and HOBT (1.07 g. 7.92 mmole) in 10 mL of DMF and 0.99 mL of NMM (9.0 mmole) is charged with EDAC (0.61 g, 3.17 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$,

and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with EtOAc to give **75**.

(2R)-*N*-Hydroxycarboxamidomethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (11). The benzyl hydroxamic acid **75** (700 mg, 1.39 mmole) is taken in 10 mL of EtOH and the mixture is charged with 10% palladium on carbon (70 mg) and stirred under one atmosphere of hydrogen for 1.5 hr. The mixture is filtered through celite and concentrated to give a solid, which is purified by flash chromatography (silica, 4% formic acid in EtOAc) to give **11**.

Example 12

Synthesis of (2R)-N-Hydroxycarboxamidomethyl decanoic acid amide of 1-N-n-butyl-caprolactam-(3S)-amine (12)



(3S)-*tert*-Butoxycarbonylamino-*N*-*n*-butyl-caprolactam (76). (3S)-*tert*-Butoxy-carbonylamino caprolactam **33** (2.0 g, 8.8 mmole) is dissolved in DMF (8 mL). Potassium *tert*-butoxide (1.25 g, 10.6 mmole) is added dropwise, and stirred for 50 min. 1-Bromo butane (1.13 mL, 10.6 mmole) is then added. The resulting mixture is stirred for 3 hr, at which time the reaction is quenched with water and extracted with ethyl acetate. The organic layer is then washed with 1N HCl, H_2O, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with hexanes:EtOAc (1:1) to give **76**.

(2R)-carbo-*tert*-butoxymethyl decanoic acid amide of *N*-*n*-butyl-caprolactam-(3S)-amine (78). **76** (2.37g, 8.3 mmole) is dissolved in 15 mL of methylene chloride, cooled to 0°C, and trifluoroacetic acid (15 mL) is added slowly. The mixture is stirred for 2 hr at room temperature and evaporated to give **77**.

The caprolactam amine **77** is mixed with **62** (1.5 g, 5.2 mmole), HOBT (3.36 g, 24.9 mmole), EDAC (1.92 g, 10 mmole) in 15 mL of DMF and 2.3 mL (20.8 mmole) of NMM at 0°C, and stirred for 17 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, H_2O, 1N NaOH, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with EtOAc to give **78**.

(2R)-*O*-Benzylhydroxycarboxamidomethyl decanoic acid amide of *N*-*n*-butyl-caprolactam-(3S)-amine (**80**). **78** (1.4 g, 3.1 mmole) is dissolved in 15 mL of methylene chloride and cooled to 0°C and trifluoroacetic acid (15 mL) is added slowly. The mixture is stirred for 1.5 hr at room temperature and evaporated to give **79**.

A mixture of the crude acid **79** (1.5 g, 3.1 mmole), *O*-benzyl hydroxylamine hydrochloride (0.6 g, 3.72 mmole) and HOBT (1.26 g, 9.3 mmole) in 10 mL of DMF and 1.16 mL of NMM (10.5 mmole) is charged with EDAC (0.71 g, 3.72 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, 1N NaOH, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with EtOAc to give **80**.

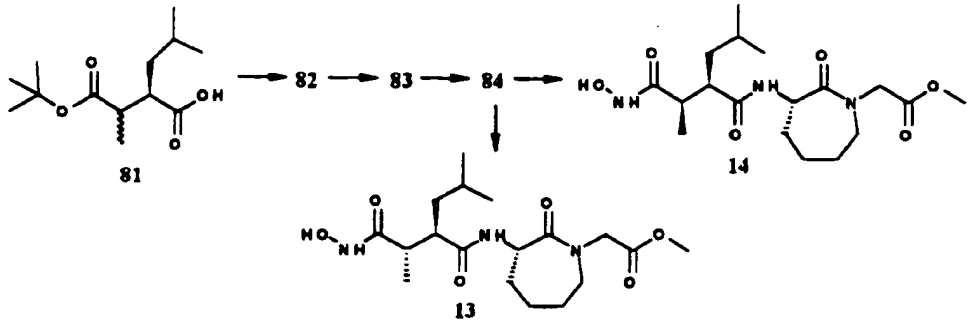
(2R)-*N*-Hydroxycarboxamidomethyl decanoic acid amide of 1-*N*-*n*-butyl-caprolactam-(3S)-amine (**12**). The benzyl hydroxamic acid **80** (900 mg, 1.8 mmole) is taken in 10 mL of EtOH and the mixture is charged with 10% palladium on carbon (90 mg) and stirred under one atmosphere of hydrogen for 2 hr. The mixture is filtered through celite and concentrated to give a solid, which is purified by flash chromatography (silica, 2% formic acid in EtOAc). The product is then crystallized from ethyl acetate to give **12**.

Example 13

(2R)-Isobutyl-(3S)-[N-hydroxycarboxamido]-butanoic acid amide of (1N)-carbomethoxymethyl-caprolactam-(3S)-amine (**13**)

Example 14

(2R)-Isobutyl-(3R)-[N-hydroxycarboxamido]-butanoic acid amide of (1N)-carbomethoxymethyl-caprolactam-(3S)-amine (14)



(2R)-Isobutyl-(3R,S)-carbo-*tert*-butoxybutanoic acid amide of (1*N*)-carbomethoxymethylcaprolactam-(3S)-amine (82). A mixture of acids **81** (3R:3S = 8:1; 720 mg, 2.95 mmole), **55** (767 mg, 3.84 mmole) and HOBT (1.39 g. 10.33 mmole) in 4 mL of DMF and 4 mL of NMM is charged with EDAC (737 mg, 3.84 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give a crude solid which is chromatographed over flash silica with hexanes:EtOAc (3:1 → 1:2) to give **82** (3R:3S = 8:1).

(2R)-Isobutyl-(3R,S)-[*O*-benzyl-*N*-hydroxycarboxamido]-butanoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine (84). The esters **82** (3R:3S = 8:1; 560 mg, 1.31 mmole), are dissolved in 4 mL of CH_2Cl_2 under argon and to this mixture is added 4 mL of trifluoroacetic acid via syringe. The mixture is stirred for 30 min. at which time the mixture is concentrated to give **83**.

A mixture of the resulting crude acid **83** (560 mg, 1.51 mmole), *O*-benzyl hydroxylamine hydrochloride (314 mg, 1.96 mmole) and HOBT (917 mg. 6.80 mmole) in 4 mL of DMF and 4 mL of NMM is charged with EDAC (376 mg, 1.96 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated. The crude solid is filtered through a plug of silica gel and then recrystallized to give the **84** (3R) diastereomer. The mother liquor is then chromatographed through silica gel with hexanes:EtOAc (1:1 → 0:1) to give **84** (3S).

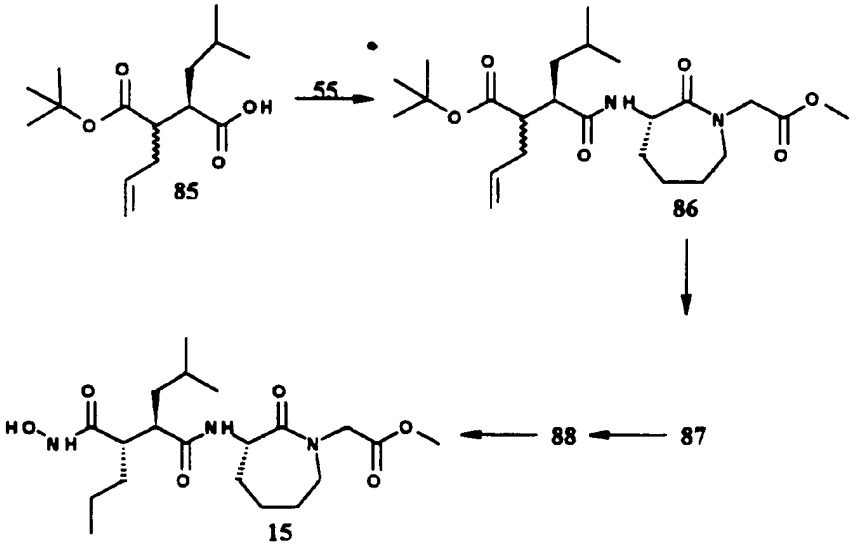
(2R)-Isobutyl-(3S)-[*N*-hydroxycarboxamido]-butanoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine (13). The benzyl hydroxamic acid **84** (3S) (45 mg, 0.095 mmole) is taken in 1 mL 95% EtOH and the mixture is charged with

5 mg of 10% palladium on carbon and stirred under one atmosphere of hydrogen for 12 hours. The mixture is then filtered through celite and concentrated to give a crude oil which is crystallized from EtOAc:EtOH (20:1) to give **13**.

(2R)-Isobutyl-(3R)-[*N*-hydroxycarboxamido]-butanoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine (**14**). The benzyl hydroxamic acid **84** (3R) (100 mg, 0.211 mmole) is taken in 1.5 mL 95% EtOH and the mixture is charged with 10% palladium on carbon (10 mg) and stirred under one atmosphere of hydrogen for 15 hours. The mixture is then filtered through celite and concentrated to give **14**.

Example 15

Synthesis of (2R)-Isobutyl-(3S)-[N-hydroxycarboxamido]-hexanoic acid amide of (1N)-carbomethoxymethyl-caprolactam-(3S)-amine (**15**)



(2R)-Isobutyl-(3S)-[carbo-*tert*-butoxy]-hex-5-enoic acid amide of (1*N*)-carbo-methoxymethylcaprolactam-(3S)-amine (**86**). A mixture of acid **85** (1.91 g, 7.07 mmole), **55** (1.84 g, 9.20 mmole) and HOBT (2.77 g, 20.5 mmole) in 2 mL of DMF and 2 mL of NMM is charged with EDAC (2.04 g, 10.6 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give a crude solid which chromatographed over flash silica with hexanes : EtOAc (3:1 → 1:2) to give **86**.

(2R)-Isobutyl-(3R,S)-carboxyhex-5-enoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine (**87**). Trifluoroacetic acid (15 mL) is added via syringe to a solution of *tert*-butyl esters **86** (3R:3S = 2:3; 1.51g, 3.34 mmole) in 15 mL of CH_2Cl_2 under argon and the resulting mixture is stirred for 1 hr at room

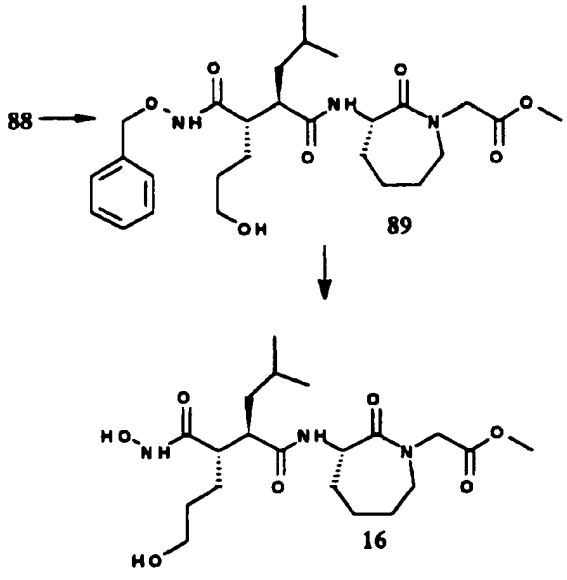
temperature. The material is then concentrated under vacuum to give **87** as a diastereomeric mixture (3R:3S = 2:3) which is carried forward without purification.

(2R)-Isobutyl-(3S)-[*O*-benzyl-*N*-hydroxycarboxamido]-hex-5-enoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine (**88**). A mixture of acids **87** (3R:3S = 2:3; 1.32 g, 3.34 mmole), *O*-benzyl hydroxylamine hydrochloride (0.693 g, 4.34 mmole) and HOBT (1.26 g. 9.35 mmole) in 7 mL of DMF and 7 mL of NMM is charged with EDAC (0.962 mg, 5.01 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated to give a crude solid which is filtered through a plug of silica gel with EtOAc and then recrystallized 2x from hexanes:EtOAc (2:1) to give **88**.

(2R)-Isobutyl-(3S)-[*N*-hydroxycarboxamido]-hexanoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine (**15**). The benzyl hydroxamic acid **88** (176 mg, 0.351 mmole) is taken in 1 mL of 95% EtOH and the mixture is charged with 30 mg of 10% palladium on carbon and stirred under one atmosphere of hydrogen for 12 hours. The mixture is then filtered through celite and concentrated to give **15**.

Example 16

(2R)-Isobutyl-(3S)-[N-hydroxycarboxamido]-6-hydroxyhexanoic acid amide of (1N)-carbomethoxymethyl-caprolactam-(3S)-amine (**16**)



(2R)-Isobutyl-(3S)-[*O*-benzyl-*N*-hydroxycarboxamido]-6-hydroxyhexanoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine (**89**). The starting olefin **88** (3R:3S = ~1:1; 240 mg, 0.479 mmol) is taken in 5 mL of dry THF under argon and cooled to 0°C at which time 9-borabicyclononane ("9-BBN") (3.45 mL, 0.5M in THF,

1.73 mmol) is added via syringe and the solution is stirred at 0°C for 2 hr at which time NaOH (2 mL, 1M) and H_2O_2 (2 mL, 30%) are added and the resulting solution stirred for 5 min. The mixture is then partitioned between EtOAc and water and the organic layer is washed with sat. NH_4Cl and brine, dried over $MgSO_4$, filtered and evaporated to give a crude oil. This diastereomeric mixture is chromatographed 2x over flash silica with EtOAc:EtOH (20:1) to give the 1S-**89**.

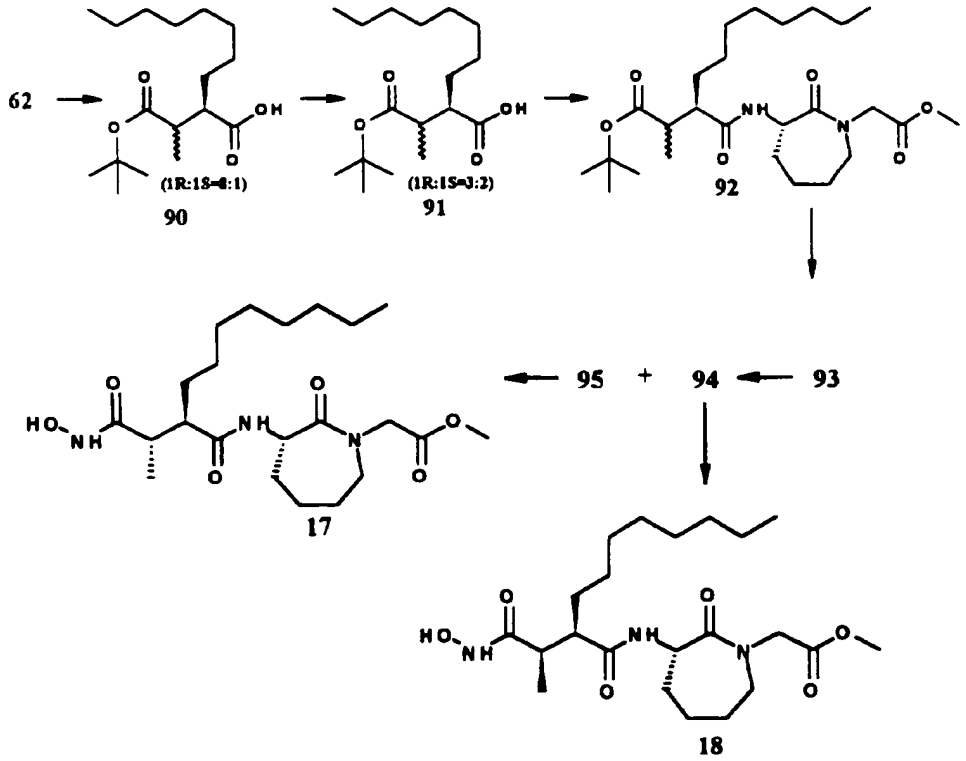
(2R)-Isobutyl-(3S)-[*N*-hydroxycarboxamido]-6-hydroxyhexanoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine (**16**). The benzyl hydroxamic acid **89** (45 mg, 0.351 mmole) is taken in 1 mL of 95% EtOH and the mixture is charged with 10 mg of 10% palladium on carbon and stirred under one atmosphere of hydrogen for 12 hours. The mixture is then filtered through celite and concentrated to give **16**.

Example 17

Synthesis of (2R)-[(1S)-N-hydroxycarboxamido]-ethyldecanoic acid amide of 1N-(carbomethoxy-methyl)-caprolactam-(3S)-amine (**17**)

Example 18

Synthesis of (2R)-[(1R)-N-hydroxycarboxamido]-ethyldecanoic acid amide of 1N-(carbomethoxy-methyl)-caprolactam-(3S)-amine (**18**)



(2R)-[(1R)-Carbo-*tert*-Butoxy]-ethyl decanoic acid (90). The starting acid **62** (4.5 g, 15.73 mmole) is dissolved in 5 mL of dry THF under an argon atmosphere and cooled to -78°C at which time lithium hexamethyldisilazane (39.3 mL, 1 M in THF, 39.3 mmole) is added via syringe and the mixture is stirred for 20 min. The mixture is allowed to warm to 0°C and then recooled to -78°C. Methyl iodide (1.08 mL, 17.3 mmole) is then added slowly via syringe and the resulting mixture is stirred for 2 hr at -78°C and 30 min. at 0°C, at which time the reaction is quenched with saturated NH_4Cl, partitioned between EtOAc and water and layers separated. The organic layer is washed with NH_4Cl, and brine, dried over $MgSO_4$, filtered and evaporated to give 5.81 g of crude oil which is chromatographed over flash silica with hexane:EtOAc (2:1) to give **90**.

(2R)-[(1R)-Carbo-*tert*-Butoxy]-ethyl decanoic acid (91). The predominately (1R) acid **90** (1R:1S = 8:1, 5 g, 15.7 mmole) is dissolved in 5 mL of dry THF under an argon atmosphere and cooled to -78°C. At which time lithium diisopropylamine (47 mL, 2 M in THF, 47 mmole) is added via syringe and the mixture is stirred for 16 hr at -78°C to 22°C as the dry ice-acetone bath warmed to room temperature. The mixture is then recooled to -78°C, quenched with 15 mL of methanol (78 mmole) and poured into NH_4Cl. After warming up to room temperature, the reaction mixture is partitioned between EtOAc and water and layers separated. The organic layer is washed with 1 N HCl, and brine, dried over $MgSO_4$, filtered and evaporated to give 5.81 g of crude oil which is chromatographed over flash silica with hexane:EtOAc (2:1) to give **91** (1S:1R = 2:3).

(2R)-[(1R,S)-Carbo-*tert*-butoxy]-ethyldecanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine (92). A mixture of acids **91** (1R:1S = 3:2, 1.5 g, 5.00 mmole), the amine **55** (1.5 g, 7.50 mmole) and HOBT (1.53 g. 10.00 mmole) in 10 mL of DMF and 1.1 mL of NMM is charged with EDAC (1.15 mg, 6.00 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to a crude solid which is chromatographed over flash silica with hexanes:EtOAc (1:1) to give **92** (1R:1S = 3:2).

(2R)-[(1R)-*O*-Benzyl-*N*-hydroxycarboxamido]-ethyldecanoic acid amide of 1*N*-(carbomethoxymethyl)-caprolactam-(3S)-amine (94). (2R)-[(1S)-*O*-Benzyl-*N*-hydroxy-carboxamido]-ethyldecanoic acid amide of 1*N*-(carbomethoxymethyl)-caprolactam-(3S)-amine (**95**). The esters **92** (1R:1S = 3:2, 1.40 g, 2.90 mmole), are dissolved in 7 mL of CH_2Cl_2 under argon and to this mixture is added 7 mL of trifluoroacetic acid via

syringe. The mixture is stirred for 2 hr at which time the mixture is concentrated to give **93**.

A mixture of the resulting crude acid **93** (1.27 g, 2.86 mmole), *O*-benzyl hydroxylamine hydrochloride (456 mg, 2.86 mmole) and HOBT (875 mg. 572 mmole) in 5 mL of DMF and 941 µL of NMM is charged with EDAC (658 mg, 3.43 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated. The crude solid is then chromatographed through silica gel with hexanes:EtOAc (1:2) to give **94** and **95**.

(2R)-[(1S)-*N*-hydroxycarboxamido]-ethyldecanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine (17). The benzyl hydroxamic acid **95** (260 mg, 0.489 mmole) is taken in 6 mL EtOH and the mixture is charged with 10% palladium on carbon (60 mg) and stirred under one atmosphere of hydrogen for 45 min. The mixture is then filtered through celite, concentrated and then recrystallized from EtOAc to give **17**.

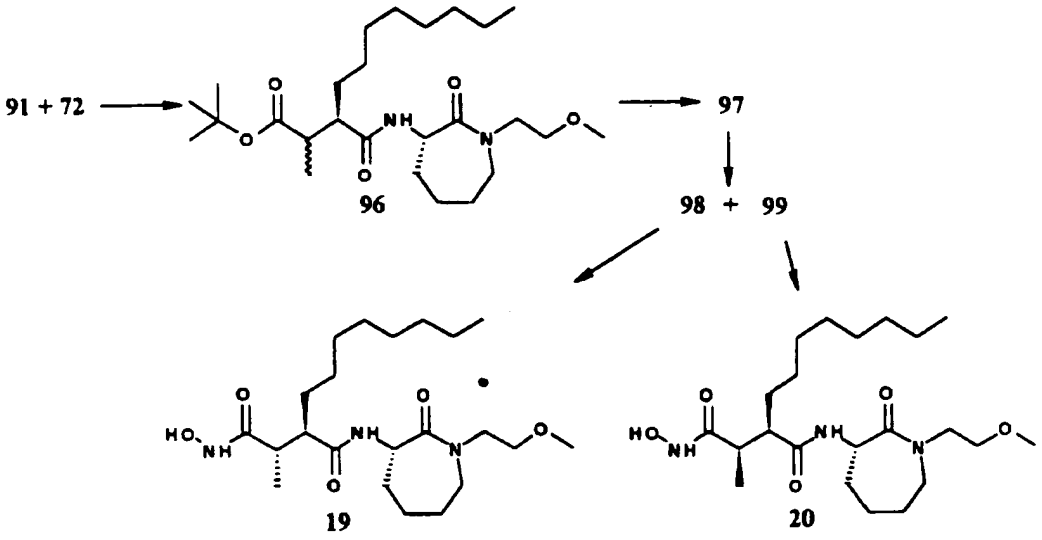
(2R)-[(1R)-*N*-hydroxycarboxamido]-ethyldecanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine (18). The benzyl hydroxamic acid **94** (300 mg, 0.565 mmole) is taken in 6 mL of EtOH and the mixture is charged with 10% palladium on carbon (60 mg) and stirred under one atmosphere of hydrogen for 45 min. The mixture is then filtered through celite, concentrated and then recrystallized from EtOAc to give **18**.

Example 19

Synthesis of (2R)-[(1S)-N-Hydroxycarboxamido]-ethyl decanoic acid amide of N-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (19)

Example 20

Synthesis of (2R)-[(1R)-N-Hydroxycarboxamido]-ethyl decanoic acid amide of N-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (20)



(2R)-[(1R,S)-*Tert*-Butylcarboxy]-ethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (96). (3S)-Amino-*N*-[(2-methoxy)ethyl]-caprolactam **72** (2.0 g, 10.78 mmole) is mixed with the decanoic acid **91** (1.5 g, 5.2 mmole), HOBT (3.65 g, 27 mmole), EDAC (2.07 g, 10.8 mmole) in 15 mL of DMF and 3 mL (27 mmole) of NMM at 0°C, and stirred for 17 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, H_2O, 1N NaOH, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with hexane:EtOAc (1:1) to give **96**.

(2R)-[(1S)-*O*-Benzylhydroxycarboxamic]-ethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (98). (2R)-[(1S)-*tert*-butyl carboxy]-ethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine **96** (1.95 g, 4.1 mmole) is dissolved in 20 mL of methylene chloride and cooled to 0°C, trifluoroacetic acid (20 mL) is added slowly. The mixture is stirred for 2 hr at room temperature and evaporated to give **97**. A mixture of the crude acid **97** (2.3 g, 4.1 mmole), *O*-benzyl hydroxylamine hydrochloride (0.78 g, 4.92 mmole) and HOBT (1.66 g. 12.3 mmole) in 15 mL of DMF and 1.54 mL of NMM (14 mmole) is charged with EDAC (0.94 g, 4.92 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is washed with 1N HCl, 1N NaOH, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with EtOAc:CH_2Cl_2:CH_3OH = 10:10:1) to give the

desired product as a diastereomeric mixture (1R:1S = 3:2). Separation of single isomers is achieved by crystallization from EtOAc:hexane (3:2) to give 1S-**98**.

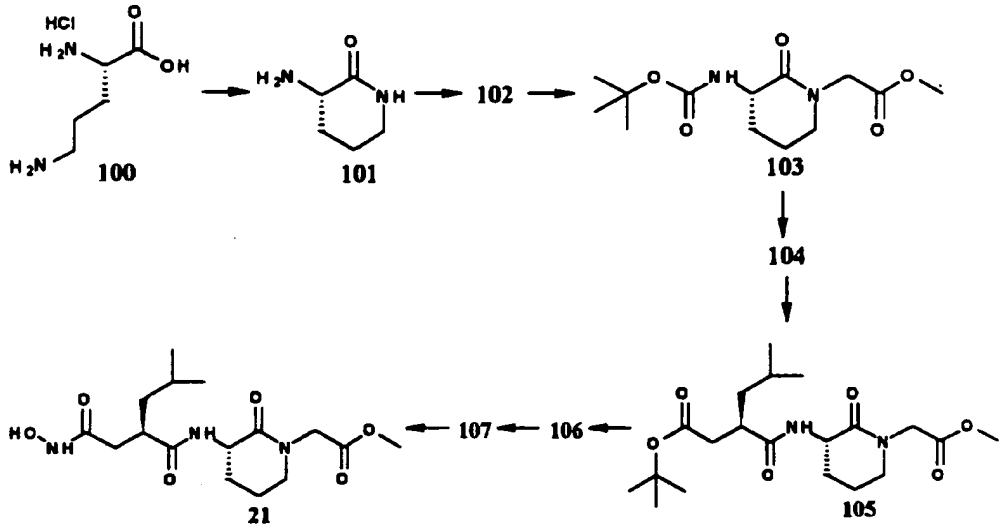
(2R)-[(1R)-*O*-Benzylhydroxycarboxamic]-ethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (**99**). The mother liquor is concentrated to give 1R-**99**.

(2R)-[(1S)-*N*-Hydroxycarboxamido]-ethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (**19**). The benzyl hydroxamic acid **98** (280 mg, 0.54 mmole) is taken in 8 mL of EtOH and the mixture is charged with 10% palladium on carbon (28 mg) and stirred under one atmosphere of hydrogen for 2 hr. The mixture is filtered through celite and concentrated to give a solid, which is purified by crystallization from EtOAc:CH_3OH (10:1) to give **19**.

(2R)-[(1R)-*N*-Hydroxycarboxamido]-ethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine (**20**). The benzyl hydroxamic acid **99** (670 mg, 1.3 mmole) is taken in 10 mL of EtOH and the mixture is charged with 10% palladium on carbon (66 mg) and stirred under one atmosphere of hydrogen for 3.5 hr. The mixture is filtered through celite and concentrated to give a solid, which is purified by crystallization from EtOAc to give **20**.

Example 21

Synthesis of (2R)-Isobutyl-3-(N-hydroxycarboxamido)-propanoic acid amide of N-(carbomethoxymethyl)-valerolactam-(3S)-amine



(3S)-Amino-valerolactam (**101**). To a 1 L 3 neck round bottom flask equipped with a condenser, thermometer, magnetic stirrer and argon inlet, is added L-ornithine hydrochloride 100 (15 g, 89 mmole), acetonitrile (280 mL) and hexamethyldisilazane (100 mL, 620 mmole). The mixture is heated to reflux for two days, then cooled to

room temperature and poured into 500 mL of methanol. The solvents are removed by rotary evaporation. The residue is taken up in 250 mL of methylene chloride, then treated with activated carbon, filtered and concentrated to a solid **101**. The compound is used without purification.

(3S)-*tert*-Butoxycarbonylamino valerolactam (**102**). (3S)-Amino-valerolactam (crude, 10.1 g, 88.4 mmole) is mixed with di-*tert*-butyl-dicarbonate **101** (19.9 g, 88.4 mmole) in 50 mL of methyl sulfoxide. The mixture is stirred overnight. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 0.1N HCl, H_2O, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with hexanes:EtOAc (1:1) to give **102**.

3S)-*tert*-Butoxycarbonylamino-*N*-carbomethoxymethyl-valerolactam (**103**). (3S)-*tert*-Butoxycarbonylamino valerolactam **102** (1.85 g, 8.63 mmole) is dissolved in THF (5 mL) and cooled to -78°C. Lithium bis(trimethylsilyl)amide (11 mL, 10.4 mmole, 1 M in THF) is added dropwise. After 10 min, methyl bromoacetate (1.1 mL, 11.2 mmole) is added. The resulting mixture is stirred at -78°C for 2 hr, and at room temperature for 1 hr. The reaction is quenched with NH_4Cl, extracted by ethyl acetate. The organic layer is then washed with 0.1N HCl, H_2O, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with hexanes:EtOAc (1:1) to give **103**.

[(2R)-Isobutyl-3-carbo-*tert*-butoxy]-propanoic acid amide of *N*-carbomethoxymethyl-valerolactam-(3S)-amine (**105**). (3S)-*tert*-Butoxycarbonylamino-*N*-carbomethoxymethyl-valerolactam **103** (2.1 g, 7.3 mmole) is dissolved in 20 mL of methylene chloride, trifluoroacetic acid (20 mL) is added slowly. The mixture is stirred for 2 hr and evaporated to give **104**. The valerolactam amine **104** is mixed with (2R)-isobutyl-3-carbo-*tert*-butoxy-propanoic acid **23** (2.52g, 11 mmole), HOBT (3.0 g. 21.9 mmole), EDAC (2.1 g, 11 mmole) in 10 mL of DMF and 4.2 mL (29.2 mmole) of triethylamine at 0°C, and stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with EtOAc to give **105**.

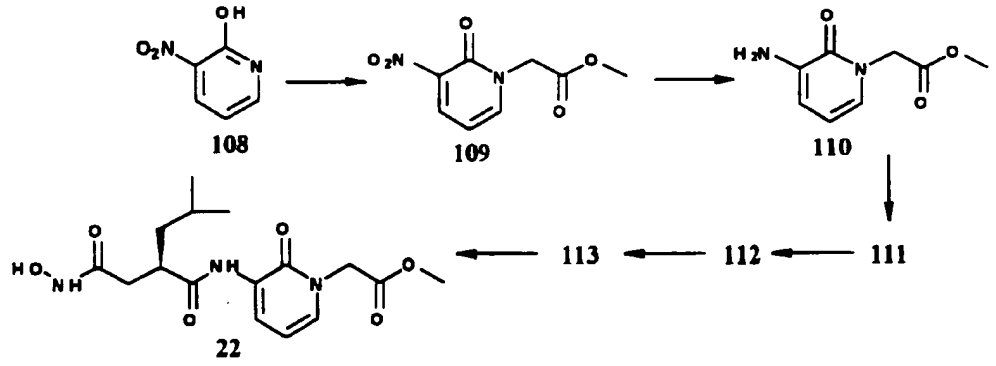
[(2R)-Isobutyl-3-(*O*-benzyl-*N*-hydroxycarboxamido)]-propanoic acid amide of *N*-(carbomethoxymethyl)-valerolactam-(3S)-amine (**107**). [(2R)-Isobutyl-3-*tert*-butyl-carboxy]-propanoic acid amide of *N*-carbomethoxymethyl-valerolactam-(3S)-amine **105** (2.2 g, 5.5 mmole) is dissolved in 10 mL of methylene chloride and trifluoroacetic acid (10 mL) is added slowly. The mixture is stirred for 2 hr and evaporated to give **106**. A mixture of the crude acid **106** (2.8 g, 5.5 mmole), *O*-benzyl hydroxylamine hydrochloride (1.96 g, 12.3 mmole) and HOBT (3.3 g. 24.6 mmole) in 10 mL of DMF

and 2 mL of NMM (28.7 mmole) is charged with EDAC (2.0 g, 10.7 mmole) and the reaction is stirred for 15 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which chromatographed over flash silica with hexanes:EtOAc (1:1 → 1:4) to give **107**.

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propanoic acid amide of *N*-(carbomethoxymethyl)-valerolactam-(3S)-amine (21). The benzyl hydroxamic acid **107** (850 mg, 1.90 mmole) is taken in 10 mL EtOH and the mixture is charged with 10% palladium on carbon (85 mg) and stirred under one atmosphere of hydrogen for 1 hr. The mixture is then filtered through celite and concentrated to an oil. The crude product is purified on a silica gel column using 2% formic acid in ethyl acetate as the eluent to give **21**.

Example 22

Synthesis of [(2R)-Isobutyl-3-(N-hydroxylcarboxamido)]-propanoic acid amide of 2-oxo-3-amino-N-(carbomethoxymethyl)-pyridinone (22)



2-Oxo-3-nitro-*N*-(carbomethoxymethyl)-pyridinone (109). 2-Hydroxy-3-nitropyridine **108** (10 g, 71.38 mmole) is mixed with pulverized potassium carbonate (10.9 g, 78.5 mmole) and 30 mL of DMF. After 10 min, methyl bromoacetate (10.4 mL, 107 mmole) is added. The resulting mixture is stirred at room temperature for 4.5 hr. The reaction is quenched by water and extracted by ethyl acetate. The organic layer is then washed with brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with EtOAc to give **109**.

2-Oxo-3-amino-*N*-(carbomethoxymethyl)-pyridinone (110). The pyridinone **109** (3.0 g, 14.1 mmole) is taken in 45 mL of EtOH and the mixture is charged with 10% palladium on carbon (0.3 g) and stirred under one atmosphere of hydrogen for 17 hr. The mixture is then filtered through celite and concentrated to an oil. The crude product is chromatographed over flash silica with hexane:ethyl acetate (1:1 to 100% EtOAc) to give **110**.

[(2R)-Isobutyl-3-carbo-*tert*-butoxy-propanoic acid amide of 2-oxo-3-amino-*N*-(carbomethoxymethyl)-pyridinone (111). The acid **23** (2.5 g, 11 mmole) is dissolved in 15 mL of THF and cooled to -15°C. NMM (1.3 mL, 11 mmole) and isobutylchloroformate (11 mmole, 1.6 mL) are added. After stirring for 30 min., NMM (1.3 mL, 11 mmole) and **110** (2.0 g, 11 mmole) are added. The reaction mixture is stirred for 17 hr and then quenched with 1N HCl and extracted with EtOAc. The organic layer is washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with EtOAc to give **111**.

[(2R)-Isobutyl-3-*O*-Benzylhydroxylcarboxamido]-propanoic acid amide of 2-oxo-3-amino-*N*-(carbomethoxymethyl)-pyridinone (113). The ester **111** (0.8 g, 2 mmole) is dissolved in 5 mL of methylene chloride and trifluoroacetic acid (5 mL) is added slowly at 0°C. The mixture is stirred for 2 hr and evaporated to give **112**. The crude acid **112** is mixed with *O*-benzylhydroxylamine hydrochloride (0.5 g, 3.0 mmole), HOBT (0.81 g, 6 mmole), EDAC (0.58 g, 3 mmole) in 10 mL of DMF and 0.77 mL (7 mmole) of NMM at 0°C, and stirred for 17 hr at room temperature. The reaction is then partitioned between water and EtOAc. The organic layer is then washed with 1N HCl, $NaHCO_3$, and brine, dried over $MgSO_4$, filtered and evaporated to give an oil which is chromatographed over flash silica with hexane:EtOAc (2:3 to 1:4) to give **113**.

[(2R)-Isobutyl-3-(*N*-hydroxylcarboxamido)]-propanoic acid amide of 2-oxo-3-amino-*N*-(carbomethoxymethyl)-pyridinone (22). The benzyl hydroxamic acid **113** (180 mg, 0.4 mmole) is taken in 5 mL of EtOH and the mixture is charged with 10% palladium on carbon (20 mg) and stirred under one atmosphere of hydrogen for 6 hours. TLC (EtOAc) indicates persistence of a small amount of starting material left. More palladium on carbon (18 mg) is added, **113** is and hydrogenated for another 20 min. The mixture is then filtered through celite and concentrated to give a semisolid, which is purified by preparative TLC (silica, EtOAc) to give **22**.

Example A

A tablet composition for oral administration, according to the present invention, is made comprising:

Component	Amount
•(2R)-[(1S)-*N*-Hydroxycarboxamido]-ethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine[1]	15. mg
•Lactose	120. mg
•Maize Starch	70. mg

•Talc	4. mg
•Magnesium Stearate	1. mg

1: The hydroxamic acid prepared according to Example 19. Other compounds having a structure according to Formula (I) are used with substantially similar results.

Example B

A capsule for oral administration, according to the present invention, is made comprising:

Component	Amount (%w/w)
•(2R)-[(1S)-N-hydroxycarboxamido]-ethyldecanoic acid amide of 1N-(carbomethoxy-methyl)-caprolactam-(3S)-amine[2]	15%
•Polyethylene glycol	85%

2: The hydroxamic acid prepared according to Example 17. Other compounds having a structure according to Formula (I) are used with substantially similar results.

Example C

A saline-based composition for local administration, according to the present invention, is made comprising:

Component	Amount (%w/w)
•(2R)-Isobutyl-(3S)-[N-hydroxycarboxamido]-6-hydroxyhexanoic acid amide of (1N)-carbomethoxymethyl-caprolactam-(3S)-amine[3]	10%
•Ethanol	10%
•Saline	80%

3: The hydroxamic acid prepared according to Example 16. Other compounds having a structure according to Formula (I) are used with substantially similar results.

Example D

A human female subject weighing 60 kg (132 lbs), suffering from rheumatoid arthritis, is treated by a method of this invention. Specifically, for 2 years, a tablet containing 50 mg of (2R)-*N*-hydroxycarboxamidemethyldecanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine (prepared according to Example 9) is administered orally to said subject.

At the end of the treatment period, the patient is examined and is found to have reduced inflammation, and improved mobility without concomitant pain.

Other compounds having a structure according to Formula (I) are used with substantially similar results.

Example E

A human male subject weighing 90 kg (198 lbs), suffering from osteoarthrits, is treated by a method of this invention. Specifically, for 5 years, a capsule containing 70 mg of (2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propanoic acid amide of *N*-(carbomethoxymethyl)-valerolactam-(3S)-amine (made according to Example 21) is administered daily to said subject.

At the end of the treatment period, the patient is examined via orthoscopy, and found to have no further advancement of erosion/fibrillation of the articular cartilage.

Other compounds having a structure according to Formula I are used with substantially similar results.

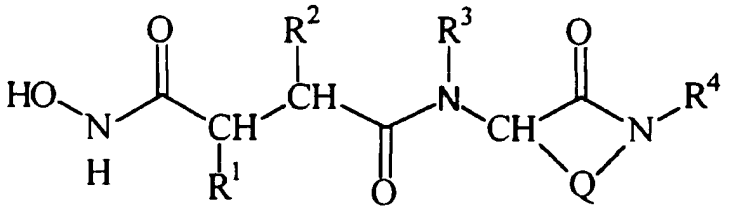
Example F

A human male subject weighing 90 kg (198 lbs), suffering from corneal ulcerations, is treated by a method of this invention. Specifically, for 2 months, a saline solution containing 10 mg of (2R)-N-Hydroxycarboxamidomethyl heptanoic acid amide of N-(carbomethoxymethyl)-caprolactam-(3S)-amine (made according to Example 8) is administered to said subject's affected eye twice-daily.

Other compounds having a structure according to Formula I are used with substantially similar results.

What is Claimed is:

1. A compound having a structure according to Formula I



(I)

wherein

(A) (1) (a) R^1 is hydrogen; alkyl; heteroalkyl; alkenyl; a heterocyclic ring; a carbocyclic ring; alkoxy; carbocycle-alkyl; heterocycle-alkyl; carbocycle-heteroalkyl; or heterocycle-heteroalkyl; and

(b) R^2 is hydrogen; hydroxy; alkyl; alkenyl; alkynyl; heteroalkyl; a heterocyclic ring; a carbocyclic ring; carbocycle-alkyl; heterocycle-alkyl; or -OR, where R is alkyl, alkenyl, or carbocycle-alkyl; or

(2) R^1 and R^2 together form a cycloalkyl ring having from 3 to 8 ring atoms;

(B) R^3 is hydrogen; alkyl; or carbocycle-alkyl;

(C) R^4 is

(1) alkyl;

(2) carbocycle-alkyl;

(3) $-X-C(=Y)-Z-R^5$ or $-X-CH_2-Z-R^5$, where

(a) X is covalent bond or alkyl;

(b) Y is O, S, or NH;

(c) Z is O, S, or NH; and

(d) R^5 is hydrogen; alkyl; alkenyl; carbocycle-alkyl; or aryl; or

(4) $-SO_2-R^6$, where R^6 is alkyl, carbocylce-alkyl, heterocycle-alkyl, or aryl; and

(D) Q is $-[-C(R^7)_2-]-_n$, where

(1) n is the integer 2, 3, or 4; and

(2) each R^7 is independently hydrogen or alkyl so the Q-containing heterocycle is saturated; or the R^7 moiety on two adjacent carbon atoms is a covalent bond such that the Q-containing heterocycle in Formula (I) is unsaturated;

or a pharmaceutically-acceptable salt, or biohydrolyzable alkoxyamide, acyloxyamide, or imide thereof.

2. The compound of Claim 1, wherein R^3 is hydrogen.

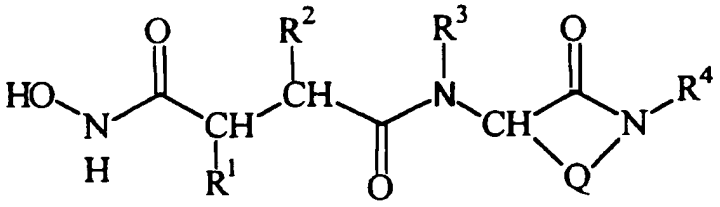
3. The compound of Claim 2, wherein R^1 is hydrogen; alkyl; or heteroalkyl; preferably R^1 is hydrogen; methyl; n-propyl; or 3-hydroxypropyl.

4. The compound of Claim 2, wherein R^2 is hydrogen; hydroxy; alkyl; alkenyl; or -OR, where R is alkyl or alkenyl; preferably wherein R^2 is C_1-C_8 alkyl or C_2-C_8 alkenyl; more preferably wherein R^2 is n-pentyl; n-octyl; or 2-methylpropyl.

5. The compound of Claim 4, wherein R^4 is carbocycle-alkyl; -X-C(=Y)-Z-R^5; or -X-CH_2-Z-R^5.

6. The compound of Claim 5, wherein:
   (a) when R^4 is carbocycle-alkyl, the alkyl is C_1-C_4 alkyl and the carbocycle is aryl;
   (b) when R^4 is -X-C(=Y)-Z-R^5, X is C_1; Y is O; Z is O; and R^5 is alkyl, carbocycle-alkyl, or aryl;
   (c) when R^4 is -X-C(=Y)-Z-R^5, X is C_1; Y is O; Z is NH; and R^5 is alkyl, carbocycle-alkyl, or aryl; or
   (d) when R^4 is -X-CH_2-Z-R^5, X is C_1; Z is O or S; and R^5 is C_1-C_3.

7. A compound having a structure according to Formula (I)



(I)

wherein

(A) (1) (i) R^1 is hydrogen; alkyl; or heteroalkyl; and
(ii) R^2 is alkyl;

(B) R^3 is hydrogen;

(C) R^4 is

(1) alkyl;

(2) carbocycle-alkyl;

(3) $-X-C(=Y)-Z-R^5$ or $-X-CH_2-Z-R^5$, where

(a) X is covalent bond or alkyl;

(b) Y is O;

(c) Z is O or NH; and

(d) R^5 is alkyl; carbocycle-alkyl; or aryl; or

(4) $-SO_2-R^6$, where R^6 is alkyl, carbocylce-alkyl, heterocycle-alkyl, or aryl; and

(D) Q is $-[-C(R^7)_2-]_n$, where n is 4 and each R^7 is independently hydrogen or alkyl so the Q-containing heterocycle is saturated; or n is 4 and the R^7 moiety on two adjacent carbon atoms is a covalent bond such that the Q-containing heterocycle in Formula (I) is unsaturated;

or a pharmaceutically-acceptable salt, biohydrolyzable amide or biohydrolyzable ester thereof.

8. The compound of Claim 7, wherein R^4 is $-X-C(=Y)-Z-R^5$.

9. The compound of Claim 8, wherein X is C_1-C_2 alkyl; Y is O; Z is O; and R^5 is methyl, benzyl, or t-butyl.

10. The compound of Claim 8, wherein X is C_1-C_2 alkyl; Y is O; Z is NH; and R^5 is methyl, benzyl or n-butyl.

11. The compound of Claim 7, wherein R^4 is $-X-CH_2-Z-R^5$.

12. The compound of Claim 11, wherein X is covalent bond or C_1-C_2; Z is O; and R^5 is C_1-C_3.

13. The compound of Claim 1, wherein the compound is selected from:

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine;

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propionic acid amide of 1*N*-(methyl-carboxamidomethyl)-caprolactam-(3S)-amine;

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propionic acid amide of 1*N*-(carbo-*tert*-butoxy-methyl)-caprolactam-(3S)-amine;

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propionic acid amide of 1*N*-Benzyl-caprolactam-(3S)-amine;

(2R)-Isobutyl-3-*N*-hydroxycarboxamidopropionic acid amide of 1*N*-(carbo-benzyloxy-methyl)-caprolactam-(3S)-amine;

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propionic acid amide of 1*N*-(*N*-benzylcarboxamidomethyl)-caprolactam-(3S)-amine;

(2R)-Isobutyl-3-*N*-hydroxycarboxamidopropionic acid amide of 1*N*-(*n*-butyl-carboxamidomethyl)-caprolactam-(3S)-amine;

(2R)-*N*-Hydroxycarboxamidomethyl heptanoic acid amide of *N*-(carbomethoxymethyl)-caprolactam-(3S)-amine;

(2R)-*N*-hydroxycarboxamidemethyldecanoic acid amide of 1*N*-(carbomethoxymethyl)-caprolactam-(3S)-amine;

(2R)-*N*-Hydroxycarboxamidomethyl decanoic acid amide of *N*-toluenesulfonyl-caprolactam-(3S)-amine;

(2R)-*N*-Hydroxycarboxamidomethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine;

(2R)-*N*-Hydroxycarboxamidomethyl decanoic acid amide of 1-*N*-*n*-butyl-caprolactam-(3S)-amine;

(2R)-Isobutyl-(3S)-[*N*-hydroxycarboxamido]-butanoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine;

(2R)-Isobutyl-(3R)-[*N*-hydroxycarboxamido]-butanoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine;

(2R)-Isobutyl-(3S)-[*N*-hydroxycarboxamido]-hexanoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine;

(2R)-Isobutyl-(3S)-[*N*-hydroxycarboxamido]-6-hydroxyhexanoic acid amide of (1*N*)-carbomethoxymethyl-caprolactam-(3S)-amine;

(2R)-[(1S)-*N*-hydroxycarboxamido]-ethyldecanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine;

(2R)-[(1R)-*N*-hydroxycarboxamido]-ethyldecanoic acid amide of 1*N*-(carbomethoxy-methyl)-caprolactam-(3S)-amine;

(2R)-[(1S)-*N*-Hydroxycarboxamido]-ethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine;

(2R)-[(1R)-*N*-Hydroxycarboxamido]-ethyl decanoic acid amide of *N*-[(2-methoxy)ethyl]-caprolactam-(3S)-amine;

(2R)-Isobutyl-3-(*N*-hydroxycarboxamido)-propanoic acid amide of *N*-(carbomethoxymethyl)-valer lactam-(3S)-amine; and

[(2R)-Isobutyl-3-(*N*-hydroxylcarboxamido)]-propanoic acid amide of 2-oxo-3-amino-*N*-(carbomethoxymethyl)-pyridinone.

14. A pharmaceutical composition comprising:

(a) a safe and effective amount of a compound of Claim 1 to Claim 13; and

(b) a pharmaceutically-acceptable carrier.

15. A method for preventing or treating a disease associated with unwanted matrixmetalloprotease activity in a human or other animal subject, the method comprising administering to said subject a safe and effective amound of a compound of Claim 1 to Claim 13.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 96/03726

A. CLASSIFICATI N OF SUBJECT MATTER
IPC 6 C07D223/12 C07D211/76 C07D213/75 A61K31/55 A61K31/445 A61K31/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. D CUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 236 872 (F. HOFFMANN - LA ROCHE AG) 16 September 1987 cited in the application see the whole document ---	1-14
Y	WO,A,90 05716 (BRITISH BIO-TECHNOLOGY LIMITED) 31 May 1990 see the whole document ---	1-14
Y	WO,A,92 17460 (SMITHKLINE BEECHAM PLC) 15 October 1992 cited in the application see the whole document ---	1-14
Y	WO,A,94 21612 (OTSUKA PHARMACEUTICAL CO., LTD.) 29 September 1994 see the whole document --- -/--	1-14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 1996	07. 08. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Hartrampf, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 96/03726

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 16982 (JAMES BLACK FOUNDATION LIMITED) 2 September 1993 see the whole document	1-14

1

INTERNATIONAL SEARCH REPORT

Inte ional application No.
PCT/US96/03726

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 15 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/03726

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-236872	16-09-87	AU-B-	588437	14-09-89
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		NO-A-	943055	11-10-94
		US-A-	5514683	07-05-96
		ZA-A-	9301193	19-08-94

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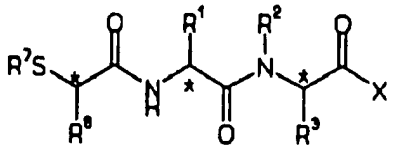
(54) Title: PEPTIDYL COMPOUNDS AND THEIR THERAPEUTIC USE AS INHIBITORS OF METALLOPROTEASES

(57) Abstract

Compounds of general formula (I) have utility as inhibitors of matrix metalloproteinases and TNF.



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Peptidyl compounds and their therapeutic use as inhibitors of metalloproteases

Field of the Invention

This invention relates to a novel class of peptidyl derivatives, to processes for their preparation, and to their use in medicine.

Background to the Invention

In normal tissues, cellular connective tissue synthesis is offset by extracellular matrix degradation, the two opposing effects existing in dynamic equilibrium. Degradation of the matrix is brought about by the action of proteinases released from resident connective tissue cells and invading inflammatory cells, and is due, in part, to the activity of at least three groups of metalloproteinases. These are the collagenases (interstitial collagenase, MMP-1; PMN collagenase, MMP-8, collagenase-3, MMP13), the gelatinases (gelatinase A, MMP-2, 72kDa-gelatinase, Type IV collagenase; gelatinase B, MMP-9, 92kDa-gelatinase, Type IV collagenase) and the stromelysins (proteoglycanase, MMP-3, stromelysin-1, transin; stromelysin-2, MMP:10; stromelysin-3, MMP:11). Normally these catabolic enzymes are tightly regulated at the level of their synthesis and secretion and also at the level of their extracellular activity, the latter through the action of specific inhibitors, such as TIMP (tissue inhibitors of metalloproteinase), which form inactive complexes with metalloproteinases, and more general proteinase inhibitors such as a_2 - macroglobulins.

The accelerated, uncontrolled breakdown of connective tissues by metalloproteinase catalysed resorption of the extracellular matrix is a feature of many pathological conditions such as rheumatoid arthritis, osteoarthritis, septic arthritis, corneal, epidermal or gastric ulceration; tumour metastasis or invasion; periodontal disease, proteinuria, coronary thrombosis associated with atherosclerotic plaque rupture and bone disease. The inhibitors claimed herein may also be useful in preventing the pathological squaelae following a traumatic injury that could lead to a permanent disability. These compounds may also have utility as a means for birth control by preventing ovulation or implantation. It can be expected that the pathogenesis of such diseases is likely to be modified in a beneficial manner by the administration of metalloproteinase inhibitors and numerous compounds have been suggested for

this purpose [for a general review see R C Wahl, *et al* Ann. Rep, Med. Chem. 25: 175-184, Academic Press Inc., San Diego (1990)].

A number of small peptide like compounds which inhibit metalloproteinases have been described. Perhaps the most notable of these are those relating to angiotensin converting enzyme (ACE) where such agents act to block the conversion of the decapeptide angiotensin I to angiotensin II, a potent pressor substance. Compounds of this type are described in EP-A-0012401. Also, related mercaptoamide peptidyl derivatives have shown ACE inhibitor activity *in vitro* and *in vivo* (H N Weller *et al* (1984), Biochem Biophys. Res. Comm., 125 (1):82-89).

TNFα is a cytokine which is produced initially as a cell-associated 28kD precursor. It is released as an active, 17kD form (D-M Jue *et al*, (1990) Biochemistry, 29:8371-8377), which can mediate a large number of deleterious effects *in vivo*. When administered to animals or humans it causes inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase responses, similar to those seen during acute infections and shock states. Chronic administration can also cause cachexia and anorexia. Accumulation of excessive TNFα can be lethal. There is considerable evidence from animal model studies that blocking the effects of TNFα with specific antibodies can be beneficial in acute infections, shock states, graft versus host reactions and autoimmune disease. TNFα is also an autocrine growth factor for some myelomas and lymphomas and can act to inhibit normal heamatopoiesis in patients with these tumours.

Preventing the production or action of TNFα is, therefore, predicted to be a potent therapeutic strategy for many inflammatory, infectious, immunological or malignant diseases. These include, but are not restricted to, septic shock, haemodynamic shock and sepsis syndrome (Mathison et al (1988) J. Clin. Invest. 81:1925-1937; Miethke et al (1992), J. Exp. Med. 175:91-98), post-ischaemic reperfusion injury, malaria (Grau et al (1989), Immunol. Rev. 112:49-70); mycobacterial infection (Barnes et al (1992) Infect. Imm. 60:1441-6), meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, autoimmune disease, rheumatoid arthritis, multiple sclerosis, radiation damage, toxicity following administration of immunosuppressive monoclonal antibodies such as OKT3 or CAMPATH-1 and hyperoxic alveolar injury.

Current clinical anti-TNFα strategies involve the use of corticosteroids such as dexamethasone, and the use of cyclosporin-A or FK506, which are non-specific inhibitors of cytokine gene transcription. Phosphodiesterase inhibitors such as pentoxyfilline have been shown to be more specific inhibitors of TNFα gene transcription (Endres S. (1991) Immunol. 72:56-60, Schandene *et al* (1992), Immunol. 76:30-34, Alegre ML, *et al* (1991); Transplantation 52:674-679, Bianco *et al* (1991) Blood 78:1205-1221). Thalidomide has also been shown to inhibit TNFα production by leucocytes (Sampajo *et al* (1991), J. Exp. Med. 173:699-703). In experimental settings, anti-TNFα monoclonal antibodies, soluble TNF receptors and soluble TNF receptor/immunoadhesins have been shown to specifically inhibit the effects of TNFα action (Bagby *et al* (1991) J. Infect. Dis. 163:83-88, Charpentier *et al.* (1991) Presse-med. 20:2009-2011, Silva *et al* (1990) J. Infect. Dis. 162:421-427; Franks *et al* (1991) Infect. Immun. 59:2609-2614, Tracey *et al* (1987) Nature 330:662-664; Fischer *et al* (1992) PNAS USA in press, Lesslauer *et al* (1991) Eur. J. Immunol. 21:2883-2886, Ashkenazi *et al* (1991) PNAS USA 88:10535-10539).

It has recently been shown that the effects of TNF are mediated by two peptides, TNFα and TNFβ. Although these peptides have only 30% homology with each other, they activate the same receptors and are encoded by immediately adjacent genes. As used herein, the term tumour necrosis factor or TNF therefore means tumour necrosis factor α and peptides having a high degrees of sequence homology with, or substantially similar physiological effects to, TNFα, for example TNFβ.

One of the objectives of the present invention is to provide compounds which substantially inhibit the release of TNF from cells, and therefore may be used in the treatment of conditions mediated by TNF. Such uses include, but are not limited to, the treatment of inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease.

Compounds which have the property of inhibiting the action of metalloproteinases involved in connective tissue breakdown such as collagenase, stromelysin and gelatinase have been shown to inhibit the release of TNF both *in vitro* and *in vivo* (AJH Gearing *et al* (1994), Nature, 370:555-557; GM McGeehan *et al* (1994),

Nature, 370:558-561: MJ Crimmin *et al*, WO 93/20047). All of these reported inhibitors contain a hydroxamic acid zinc binding group.

It is, therefore, a further objective of this invention to provide compounds which, in addition to inhibiting TNF release, also inhibit the action of MMPs, and hence may be used in the treatment of patients who suffer from conditions mediated by TNF and/or MMPs.

As appreciated by those of skill in the art the significant proportion of homology between human fibroblast collagenase, stromelysin and gelatinase leads to the possibility that a compound that inhibits one enzyme may to some degree inhibit all of them.

Compounds that inhibit collagenase, which possess structural portions akin to those of the instant invention include those encompassed by U.S.4,511,504 issued Apr. 16, 1985; U.S. 4,568,666, issued Feb 4, 1986.

Compounds of related structure that are claimed to inhibit stromelysin (proteoglycanase) are encompassed by U.S.4,771,037, issued Sept. 13, 1988.

The applicants believe that stromelysin and collagenase inhibitors have utility in preventing articular cartilage damage associated with septic arthritis. Bacterial infections of the joints can elicit an inflammatory response that may then be perpetuated beyond what is needed for removal of the infective agent resulting in permanent damage to structural components. Bacterial agents have been used in animal models to elicit an arthritic response with the appearance of proteolytic activities. See J. P. Case *et al* (1989), J. Clin. Invest., 84:1731-40; R. J. Williams *et al* (1990), Arth. Rheum., 33: 533-41.

The applicants also believe that inhibitors of stromelysin, collagenase and gelatinase will be useful to control tumour metastasis, optionally in combination with current chemotherapy and/or radiation. See L. M. Matrisian *et al* (1986), Proc. Natl. Acad. Sci., USA, 83:9413-7; S. M. Wilhelm *et al* (1987), Ibid. 84:6725-29; Z. Werb *et al* (1989), J. Cell Biol., 109:872-889; L. A. Liotta *et al* (1983), Lab. Invest., 49:636-649; R. Reich *et al* in Metatasis; Ciba Foundation Symposium, Wiley, Chicester, 1988, pp. 193-210.

Secreted proteinases such as stromelysin, collagenase and gelatinase play an important role in processes involved in the movement of cells during metastasic

tumour invasion. Indeed, there is also evidence that the matrix metalloproteinases are overexpressed in certain metastatic tumour cell lines. In this context, the enzyme functions to penetrate underlying basement membranes and allow the tumour cell to escape from the site of primary tumour formation and enter the circulation. After adhering to blood vessel walls, the tumour cells use these same metalloproteinases to pierce underlying basement membranes and penetrate other tissues, thereby leading to tumour metastasis. Inhibition of this process would prevent metastasis and improve the efficacy of current treatments with chemotherapeutics and/or radiation.

These inhibitors should also be useful for controlling periodontal diseases, such as gingivitis. Both collagenase and stromelysin activities have been isolated from fibroblasts derived from inflamed gingiva (V. J. Uitto *et al* (1981), J.Periodontal Res., 16:417-424). Enzyme levels have been correlated to the severity of gum disease; C. M. Overall *et al* (1987), J. Periodontal Res., 22:81-88.

Proteolytic processes have also been observed in the ulceration of the cornea following alkali burns (S. I. Brown *et al* (1969), Arch. Opthalmol., 81:370-373). Mercapto-containing peptides do inhibit the collagenase isolated from alkali-burned rabbit cornea (F. R. Burns *et al* (1989), Invest. Opthalmol, 30:1569-1575). Treatment of alkali-burned eyes or eyes exhibiting corneal ulceration as a result of infection with inhibitors of these metalloendoproteinases in combination with sodium citrate or sodium ascorbate and/or antimicrobials may be effective in preventing developing corneal degradation.

Stromelysin has been implicated in the degradation of structural components of the glomerular basement membrane (GBM) of the kidney, the major function of which is to restrict passage of plasma proteins into the urine (W. H. Baricos *et al* (1989), Biochem. J., 254:609-612). Proteinuria, a result of glomerular disease, is excess protein in the urine caused by increased permeability of the GBM to plasma proteins. The underlying causes of the increased GBM permeability are unknown, but proteinases including stromelysin may play an important role in glomerular diseases. Inhibition of this enzyme may alleviate the proteinura associated with kidney malfunction.

It is suggested that inhibition of stromelysin activity may prevent the rupturing of atherosclerotic plaques leading to coronary thrombosis. The tearing or rupture of

atherosclerotic plaques is the most common event initiating coronary thrombosis. Destabilisation and degradation of the connective tissue matrix surrounding these plaques by proteolytic enzymes or cytokines released by infiltrating inflammatory cells has been proposed as a cause of plaque fissuring. Such tearing of these plaques can cause an acute thrombolytic event as blood rapidly flows out of the blood vessel. High levels of stromelysin RNA message have been found to be localised to individual cells in atherosclerotic plaques removed from heart transplant patients at the time of surgery (A. M. Henney *et al* (1991), Proc. Nat'l. Acad. Sci. USA, 88:8154-8158). Inhibition of stromelysin by these compounds may aid in preventing or delaying the degradation of the connective tissue matrix that stabilises the atherosclerotic plaques, thereby preventing events leading to acute coronary thrombosis.

It is also believed that specific inhibitors of stromelysin and collagenase should be useful as birth control agents. There is evidence that expression of metalloproteinases, including stromelysin and collagenase, is observed in unfertilised eggs and zygotes and at further cleavage stages and increased at the blastocyst stage of fetal development and with endoderm differentiation (C. A. Brenner *et al* (1989), Genes & Develop., 3:848-59). By analogy to tumour invasion, a blastocyst may express metalloproteinases in order to penetrate the extracelluar matrix of the uterine wall during implantation. Inhibition of stromelysin and collagenase during these early development processes should presumably prevent normal embryonic development and/or implantation in the uterus. Such intervention would constitute a novel method of birth control. In addition there is evidence that collagenase is important in ovulation processes. In this example, a covering of collagen over the apical region of the follicle must be penetrated in order for the ovum to escape. Collagenase has been detected during this process and an inhibitor has been shown to be effective in preventing ovulation (J. F. Woessner *et al* (1989), Steroids, 54:491-499). There may also be a role for stromelysin activity during ovulation (C. K. L. Too *et al* (1984), Endocrin., 115:1043-1050).

Collagenolytic and stromelysin activity have also been observed in dystrophic epidermolysis bullosa (A. Kronberger *et al* (1982), J. Invest. Dermatol., 79:208-211; D. Sawamura *et al* (1991), Biochem. Biophys. Res. Commun., 184:1003-8).

Inhibition of metalloendoproteinases should limit the rapid destruction of connective components of the skin.

In addition to extracelluar matrix comprising structural components, stromelysin can degrade other *in vivo* substrates including the inhibitors a_1-proteinase inhibitor and may therefore influence the activities of other proteinases such as elastase (P. G. Winyard *et al* (1991), FEBS Letts., 279,1:91-94). Inhibition of the matrix metalloendoproteinases may potentiate the antiproteinase activity of these endogenous inhibitors.

From recent publications it is evident that several new enzymes of the MMP family have been identified, some of which maybe important in disease. Collagenase 3, an enzyme unique to breast carcinoma cells may have utility in breast cancer (JMP Freije *et al* (1994), J. Biol. Chem., 269 (24): 16766-16773), whilst MT-MMP, another member of the MMP family has been shown to be a key enzyme in the activation of gelatinase A (H Sato *et al* (1994), Nature, 370:61-65). Gelatinase A is an important enzyme in the growth and metastasis of tumours (such as defined above).

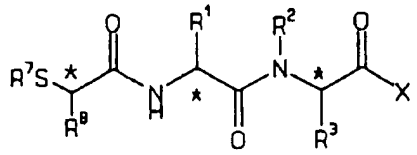
The degradation of β-Amyloid Precusor Protein (APP) has been shown to generate amyloid plaques, a major constituent of the senile plaques, found in patients with Alzheimers Disease (AD). Two recent publications have identified metalloproteinase enzymes that cleave APP to the amyloid plaque (CR Abraham *et al* (1994), Biochemistry, 33:192-199; G Huber *et al* (1994), Biochem. Biophys. Res. Comm., 201 (1):45-53).

As appreciated by those of skill in the art, the significant proportion of homology between these new enzymes and other MMPs leads to the possibility that a compound that inhibits one enzyme may to some degree inhibit these new enzymes. Therefore, inhibitors encompassed in this invention may be useful in the diseases in which these new enzymes are implicated.

SUMMARY OF THE INVENTION

The invention encompasses novel mercaptoalkylpeptidyl compounds of formula (I) which are useful inhibitors of matrix metalloproteinases and/or TNFα mediated diseases including degenerative diseases (such as defined above) and certain cancers.

In a first aspect of the invention there is provided a compound of general formula (I):



(I)

Wherein:

R^1 is C_{1-6} alkyl, C_{2-6} alkenyl, -C_{1-6} alkyl-aryl, aryl, -C_{1-6} alkyl-heteroaryl, heteroaryl or -C_{1-6} alkyl-AR^9 where A represents O,NR^9 or $S(O)_m$ where m=0-2, and R^9 is H, C_{1-4} alkyl, aryl, heteroaryl, -C_{1-4} alkyl-aryl or -C_{1-4} alkyl-heteroaryl; if $A=NR^9$ the groups R^9 may be the same or different;

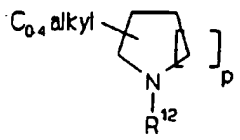
R^2 is H or C_{1-6} alkyl;

R^3 is $[Alk]_nR^6$ where Alk is C_{1-6} alkyl or C_{2-6} alkenyl and n is zero or 1;

X is NR^4R^5 where either R^4 is hydrogen or C_{1-6} alkyl optionally substituted by amino (NH_2), aryl, arylamino, protected amino, di(C_{1-6} alkyl)amino, mono(C_{1-6} alkyl)amino, CO_2H, protected carboxyl, carbamoyl, mono(C_{1-6} alkyl)carbamoyl or di(C_{1-6} alkyl)carbamoyl, and R^5 is hydrogen or C_{1-6} alkyl; or NR^4R^5 forms a ring such as pyrrolidino, piperidino or morpholino;

R^7 is hydrogen or $R^{10}CO$ where R^{10} is C_{1-4} alkyl, -C_{1-4} alkyl-aryl, -C_{1-4} alkyl-heteroaryl, cyclo(C_{3-6})alkyl, -C_{1-4} alkyl-cyclo(C_{3-6})alkyl, C_{2-6} alkenyl, -C_{2-6} alkenyl-aryl, aryl or heteroaryl;

R^8 is aryl (substituted with R^{11}), heteroaryl (optionally substituted with R^{11}), C_{1-4} alkyl-R^{11}, -C_{1-4} alkyl-aryl (substituted with R^{11}), -C_{1-4} alkyl-heteroaryl (optionally substituted with R^{11}), cyclo(C_{3-6})alkyl (optionally substituted with R^{11}), cyclo(C_{3-6})alkenyl (optionally substituted with R^{11}), -C_{1-4} alkyl-cyclo(C_{3-6})alkyl (optionally substituted with R^{11}), or any of the three groups



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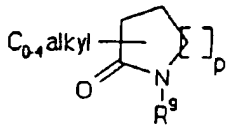
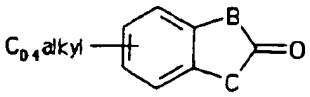




where p is 1 or 2 and B and C are independently selected from O, S, $C(R^9)_2$ and NR^9;

R^6 is AR^9, cyclo(C_{3-6})alkyl, cyclo(C_{3-6})alkenyl, C_{1-6} alkyl, -C_{1-6} alkoxy-aryl, benzyloxyaryl, aryl, heteroaryl, -C_{1-3} alkyl-heteroaryl, -C_{1-3} alkyl-aryl, -C_{1-6} alkyl-$COOR^9$, -C_{1-6} alkyl-NHR, CONHR, $NHCO_2R$, $NHSO_2R$ or NHCOR, R being defined as for R^{10};

R^{11} is SO_2R^{13}, SR^7, SR^9, COR^{13}, $N(R^9)_2$, NR^9R^{12}, OR^9, succinimido or phthalimido;

R^{12} is H or COR^9, CO_2R^9 (where R^9 is not H), $CONHR^9$ or SO_2R^9 (where R^9 is not H); and

R^{13} is OH, OC_{1-4} alkyl, O-C_{1-4} alkyl-aryl, $N(R^9)_2$ (in which the R^9s are the same or different), C_{1-4} alkyl, aryl, heteroaryl, -C_{1-4} alkyl-aryl or -C_{1-4} alkyl-heteroaryl;

the compound being in the form of a non-salt, salt, solvate or hydrate.

Preferred compounds of the invention include those in which, independently or in any combination have:

R^1 is C_{1-6} alkyl or C_{1-4} alkylAR^9 where A is $S(O)_m$,NR^9, or O and m=0,1 or 2, and

R^9 is H, C_{1-4} alkyl or aryl;

R^2 is H or C_{1-4} alkyl;

R^3 is $[Alk]_nR^6$ where n=0 or 1, Alk is C_{1-4} alkyl and R^6 is C_{1-4} alkyl, C_{1-3} alkylaryl, C_{1-3} alkylheteroaryl or AR^9;

R^4 is H;

R^5 is H or C_{1-6} alkyl;

NR^4R^5 may form a 5-7 membered ring such as a pyrrolidine, piperidine or morpholine;

R^7 is H or $R^{10}CO$ where R^{10} is C_{1-4} alkyl;

R^8 is C_{1-4} alkylR^{11}, C_{1-4} alkenylR^{11}, Cyclo(C_{3-6})alkylR^{11};

R^{11} is COR^{13}, NR^9R^{12}, $N(R^9)_2$, succinimido or phthalimido,

R^{12} is COR^9, $CO2R^9$ (provided R9 is not H), or SO_2R^9 (provided R9 is not H);

and R^{13} is OH, OC_{1-4} alkyl or $N(R^9)_2$;

Compounds of the invention have IC_{50} values below 50mM against the MMP enzymes and/or below 50mM in the whole cell assay of TNF inhibition.

It will be appreciated that the compounds according to the invention can contain one or more asymmetrically substituted carbon atoms, for example those marked with an asterisk in formula (1). The presence of one or more of these asymmetric centres in a compound of formula (1) can give rise to stereoisomers, and in each case the invention is to be understood to extend to all such stereoisomers, including enantiomers and diastereomers, and mixtures including racemic mixtures thereof.

In the formulae herein, the ~ line is used at a potential asymmetric centre to represent the possibility of *R*- and *S*- configurations, the < line and the ..... line to represent a unique configuration at an asymmetric centre.

As used in this specification, alone or in combination, the term "C_{1-6} alkyl" refers to a straight or branched chain alkyl moiety having from one to six carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, t-butyl, pentyl, hexyl and the like.

The term "C_{1-4} alkyl" refers to a straight or branched chain alkyl moiety having from one to four carbon atoms, including for example, methyl, ethyl, propyl, isopropyl, butyl, t-butyl and the like.

The term "C_{2-6} alkenyl" refers to a straight or branched chain alkyl moiety having two to six carbon atoms and having in addition one double bond, of either E or Z stereochemistry where applicable. This term would include for example, vinyl, 1-propenyl, 1- and 2- butenyl, 2- methyl-2-propenyl etc.

The term "cyclo (C_{3-6}) alkyl" refers to a saturated alicyclic moiety having from three to six carbon atoms and includes for example cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like.

The term "cyclo (C_{3-6}) alkenyl" refers to an alicyclic moiety having from three to six carbon atoms and having in addition one double bond. This term would include for example cyclopentenenyl or cyclohexenyl.

There term "aryl" means an optionally substituted phenyl or naphthyl group with the substituent(s) being selected, for example, from halogen, trifluoromethyl, C_{1-6} alkyl, alkoxy, phenyl and the like. The term "halogen" means fluorine, chlorine, bromine or iodine.

The terms "protected amino" and "protected carboxy" mean amino and carboxy groups which are protected in a manner familiar to those skilled in the art. For example, an amino group can be protected by a benzyloxycarbonyl, *tert*-butoxycarbonyl, acetyl or like groups, or in the form of a phthalimido or like group. A carboxyl group can be protected in the form of a readily cleavable ester such as the methyl, ethyl, benzyl or *tert*-butyl ester.

The term "alkoxy" refers to a straight chain or branched chain alkoxy group containing a maximum of six carbon atoms, such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, *tert*-butoxy and the like.

The term "C_{0-4} alkyl" refers to a bond or straight or branched chain alkyl moiety having from up to four carbon atoms, including for example, methyl, ethyl, propyl, isopropyl and the like.

The term "heteroaryl" refers to aromatic ring systems of five to ten atoms of which at least one atom is selected from the group, O, N or S.

Salts of compounds of formula (I) include pharmaceutically acceptable salts, for example acid addition salts derived from inorganic or organic acids, such as hydrochlorides, hydrobromides, p-toluenesulphonates, phosphates, sulphates, perchlorates, acetates, trifluoroacetates, propionates, citrates, malonates, succinates, lactates, oxalates, tartrates and benzoates.

Salts may also be formed with bases. Such salts include salts derived from inorganic or organic bases, for example alkali metal salts such as magnesium or calcium salts, and organic amine salts such as morpholine, piperidine, dimethylamine or diethylamine salts.

When the "protected carboxy" group in compounds of the invention is an esterified carboxyl group, it may be a metabolically labile ester of formula CO_2R^{14} where R^{14} may be an ethyl, benzyl, phenethyl, phenylpropyl, α- or β-naphthyl, 2,4-dimethylphenyl, 4-*tert*-butylphenyl, 2,2,2-trifluoroethyl, 1-(benzyloxy)benzyl, 1-(benzyloxy)ethyl, 2-methyl-1-propionyloxypropyl, 2,4,6-trmethylbenzyloxymethyl or pivaloyloxymethyl group.

Compounds of the general formula (I) may be prepared by any suitable method known in the art and/or by the following processes, which itself forms part of the invention.

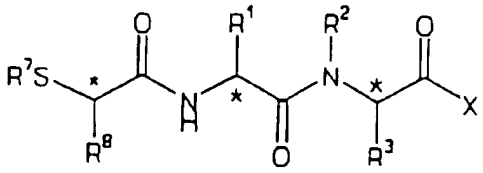
According to a second aspect of the invention, there is provided a process for preparing a compound of general formula (I) as defined above. It will be appreciated that where a particular stereoisomer of formula (I) is required, the synthetic processes described herein may be used with the appropriate homochiral starting material and/or isomers maybe resolved from mixtures using conventional separation techniques (eg. HPLC).

The compounds according to the invention may be prepared by the following process. In the description and formulae below the groups R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, R^{14}, A, B, C and X are as defined above, except where otherwise indicated. It will be appreciated that functional groups, such as amino, hydroxyl or carboxyl groups, present in the various compounds decribed below, and which it is desired to retain, may need to be in protected form before any reaction is initiated. In such instances, removal of the protecting group may be the final step in a particular reaction. Suitable protecting groups for such functionality

will be apparent to those skilled in the art. For specific details see "Protective Groups in Organic Synthesis", Wiley Interscience, T W Greene, PGM Wuts.

Thus a process for preparing compounds of general formula (I) comprises the steps of:

deprotecting (for example by hydrolysis) a compound of general formula (II)

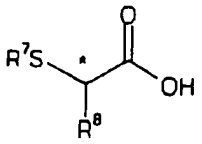


(II)

wherein R^7 represents a suitable protecting group (eg *tert* butyl or acetate).

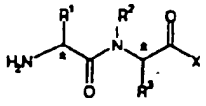
It will be appreciated that where a particular stereoisomer of formula (I) is required, this may be obtained by conventional resolution techniques such as high performance liquid chromatography. Where desired, however, appropriate homochiral starting materials may be used in the coupling reaction to yield a particular stereoisomer of formula (I). This is exemplified below.

Intermediates of general formula (II) may be prepared by coupling an acid of formula (III)



(III)

wherein R^7 and R^8 are as defined above, or an active derivative thereof, with an amine of formula (IV)

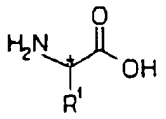


(IV)

Active derivatives of acids of formula (III) include for example acid anhydrides or acid halides, such as acid chlorides.

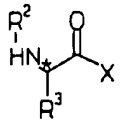
The coupling reaction may be performed using standard conditions for amination reactions of this type. Thus, the reaction may be achieved in a solvent, for example an inert organic solvent such as an ether, eg. a cyclic ether such as tetrahydrofuran, an amide eg. a substituted amide such as dimethylformamide, or a halogenated hydrocarbon such as dichloromethane at a low temperature eg. -30°C to ambient temperature, such as -20°C to 0°C, optionally in the presence of as base, eg. an organic base such as an amine, eg. triethylamine or a cyclic amine such as N-methylmorpholine. Where an acid of formula (III) is used, the reaction may additionally be performed in the presence of a condensing agent, for example a diimide such as N,N'-dicyclohexylcarbodiimide, advantageously in the presence of a triazole such as 1-hydroxybenzotriazole. Alternatively, the acid may be reacted with a chloroformate for example ethylchloroformate, prior to reaction with the amine of formula (IV).

The amine of general formula (IV) may be prepared by coupling an acid of formula (V), or an active derivative thereof



(V)

with an amine of formula (VI)



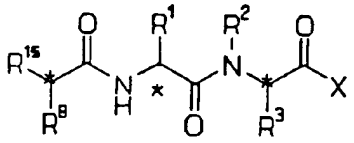
(VI)

followed by removal of any protecting groups.

Active derivates of acids for formula (V) include for example acid anhydrides or acid halides such as acid chlorides as outlined earlier.

Amino acids and their derivatives as depicted by general formulae (V) and (VI) can be obtained in chiral or racemic form. In the chiral form they provide asymmetric building blocks for the chiral synthesis of compounds of general formula (I). Many of these derivatives can be readily obtained from commercially available starting materials using methods known to those skilled in the art. (See "The Practice of Peptide Synthesis" by M. Bodanszk et al, Springer Verlag, New York, 1984, P. L. Durette, WO92/21360).

As a further extension to the present invention compounds of general formula (II) may be prepared by nucleophilic substitution of compounds of general formula (VII)



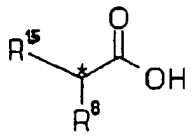
(VII)

wherein R^{15} represents a suitable leaving group (e.g. a halogen such as bromide, or an alkylsulphonate ester such as methanesulphonate) with a thiol of general formula (VIII)

$$R^7SH \qquad \text{(VIII)}$$

Wherein R^7 represents a suitable protecting group (eg. *tert*-butyl or acetate), using standard conditions known to those skilled in the art as exemplified in WO 90/05719. Thiols of general formula (VIII) may be obtained from commercially available starting materials using methods known to those skilled in the art. Many thiols of general formula (VIII) are also commercially available.

Compounds of general formula (VII) may be prepared by coupling an acid of general formula (IX)



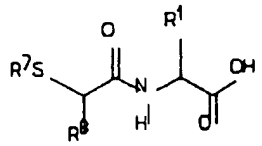
(IX)

wherein R^{15} and R^8 are as defined above (or suitably protected versions thereof) or an active derivative thereof, with an amine of formula (IV) using similar coupling conditions to those described for the preparation of compounds of formula (II).

Carboxylic acids of the structure depicted in formulae (III) and (IX) can be obtained in chiral or racemic form. Many of these derivatives can be readily obtained from commercially available starting materials using methods known to those skilled in the art (see WO 90/05719).

As a further extension to the present invention, intermediates of general formula (II) may be prepared by coupling an acid of formula (X)

(X)



wherein R^1, R^7 and R^8 are as defined above, or an active derivative thereof, with an amine of formula (VI) by the procedure described previously.

Acids of general formula (X) may in turn be prepared by coupling an acid of formula (III), or an active derivative thereof with an amine of formula (VI), where X=OH or a suitably protected derivative therof followed by removal of any protecting groups.

Active derivates of acids for formula (V) include for example acid anhydrides or acid halides such as acid chlorides as outlined earlier.

Compounds of formula (I) may also be prepared by interconversion of other compounds of formula (I). Thus, for example, a compound of formula (I) wherein R^1 is a C_{1-6} alkyl group may be prepared by hydrogenation (using palladium on carbon in suitable solvent, such as an alcohol - eg ethanol) of a compound of

formula (I) wherein R^1 is a C_{2-6} alkenyl group. A further example would include a compound of formula (I) wherein R^7 is a group R^{10} CO may be prepared by acylation (using a suitable acid chloride R^{10} COCl, in the presence of a base such as a triethylamine in a suitable solvent, such as a chlorinated solvent - eg dichloromethane) of a compound of formula (I) wherein R^7 is H.

Any mixtures of final products or intermediates obtained can be separated on the basis of the pysico-chemical differences of the constituents, in known manner, into the pure final products or intermediates, for example by chromatography, distillation, fractional crystallization, or by formation of a salt if appropriate or possible under the circumstances.

The compounds according to the invention exhibit *in vitro* inhibiting activities with respect to stromelysin, collagenase and gelatinase. Compounds according to the invention also exhibit *in vitro* inhibition of TNFα release. The activity and selectivity of the compounds may be determined by use of the appropriate enzyme inhibition test, for example as described in Example A hereinafter.

This invention also relates to a method of treatment for patients (including man and/or mammalian animals raised in the dairy, meat or fur industries or as pets) suffering from disorders or diseases which can be attributed to matrix metalloproteinases and/or TNFα as previously described, and more specifically, a method of treatment involving the administration of the matrix metalloproteinase inhibitors of formula (I) as the active constituents.

Accordingly, the compounds of formula (I) can be used among other things in the treatment of osteoarthritis and rheumatoid arthritis, and in diseases and indications resulting from the over-expression of these matrix metalloproteinases such as found in certain metastatic tumour cell lines.

As mentioned above, compounds of formula (I) are useful in human or veterinary medicine since they are active as inhibitors of TNFα and MMPs. Accordingly in another aspect, this invention concerns:

a method of management (by which is meant treatment or prophylaxis) of disease or conditions mediated by TNFα and/or MMPs in mammals, in particular in humans, which method comprises administering to the mammal an effective amount of a compound of formula (I) above, or a pharmaceutically acceptable salt thereof; and

a compound of formula (I) for use in human or veterinary medicine, particularly in the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by TNFα and/or MMPs; and

the use of a compound of formula (I) in the preparation of an agent for the management (by which is meant treatment or prophylaxis) of diseases or conditions mediated by TNFα and/or MMPs.

The disease or conditions referred to above include inflammation, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infections, shock states, graft versus host reactions and autoimmune disease; and those involving tissue breakdown such as bone resportion, inflammatory diseases, dermatological conditions, tumour growth, angiogenesis and invasion by secondary metastases, in particular rheumatoid arthritis, osteoarthritis, periodontitis, gingivitis, corneal ulceration, tumour growth, angiogenesis and invasion by secondary metastases.

For the treatment of rheumatoid arthritis, osteoarthritis, and in diseases and indications resulting from the over-expression of matrix metalloendoproteinases such as found in certain metastatic tumour cell lines or other diseases mediated by the matrix metalloendoproteinases or increased TNFα production, the compunds of formula (I) may be administered orally, topically, parenterally, by inhalation spray or rectally in dosage unit formulations containing non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, cattle, sheep, dogs, cats etc, the compounds of the invention are effective in the treatment of humans.

The pharmaceutical composition containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving

agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyeryl distearate may be employed. They may also be coated by the techniques described in the US Patents 4,256,108;4,166,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

Formulations for oral use may also be presented as hard gelatin capsules where in the active ingredient is mixed with an inert solid diluent, for example calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally occuring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters dervied from fatty acids and a hexitol such a polyoxyethylene with partial esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more

colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified, for example sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally- occuring gums, for example gum acacia or gum tragacanth, naturally-occuring phosphatides, for example soya bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example gycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavouring and colouring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be in a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles

and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds of formula (I) may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc containing the compounds of Formula (I) are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

Dosage levels of the order of from about 0.05 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above- indicated conditions (about 2.5 mg to about 7 gms per patient per day). For example, inflammation may be effectively treated by the administration of from about 0.01 to 50 mg of the compound per kilogram of body weight per day (about 0.5 mg to about 3.5 gms per patient per day).

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may vary from about 5 to about 95 percent of the total composition. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

The following Examples 1 to 79 illustrate the invention and their preparation (via the green Intermediates, as appropriate). Examples 1 to G illustrate test procedures. In the Examples, the following abbreviations are used:

RT Room temperature

DCC Dicyclohexylcarbodiimide

EDC 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride

TNF_α Tumour necrosis factor α

LPS Lipopolysaccharide

ELISA Enzyme-linked immunosorbent assay

Intermediate 1 (*RS*)-2-Bromo-4-methoxycarbonylbutanoic acid

A solution of d-methyl-*D,L*-glutamic acid (6.0 g) (preparation according to Hanby *et al*, J. Chem. Soc. (1950), 51:3239) and potassium bromide (15.5 g) in aqueous sulfuric acid (1.25 M, 100 ml) was treated at 0°C portionwise with sodium nitrite (4.0 g) over 1 h. The solution was allowed to warm to RT and was stirred over 2 h, then extracted with ethyl acetate (2 x 100 ml). The combined extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give the title compound as a colourless oil (4.5 g).

TLC R_f 0.14 (25% EtOAc-hexanes)

Similarly prepared were:

Intermediate 2 (*RS*)-2-Bromo-5-methoxycarbonylpentanoic acid

From (*RS*)-2-amino-5-methoxycarbonylpentanoic acid (1.8 g) (preparation based on the esterification procedure of Hanby *et al*, J. Chem. Soc. (1950), 51:3239) as a pale brown oil (1.95 g).

TLC R_f 0.30 (5% MeOH-CH_2Cl_2)

Intermediate 3 (*RS*)-2-Bromo-6-methoxycarbonylhexanoic acid

From (*RS*)-2-amino-6-methoxycarbonylhexanoic acid (3.93 g) (preparation based on the esterification procedure of Hanby *et al*, J. Chem. Soc. (1950), 51:3239) as a colourless oil

(4.39 g).

TLC R_f 0.26 (5% MeOH-CH_2Cl_2)

Intermediate 4 (*RS*)-2-Acetylmercapto-3-methoxycarbonylpropionic acid

A solution of potassium thiolacetate (1.48 g) in methanol (2 ml) was added to a solution of *mono*-methylmaleate (1.64 g) (prepared according the procedure exemplified in J. Am. Chem. Soc. (1986), 108:3) and the mixture was stirred overnight at RT. The solvent was removed by evaporation and the residue was partitioned between water (30 ml) and dichloromethane (30 ml), and the aqueous layer was then acidified to pH3 with 2N aqueous hydrochloric acid. The layers were separated and the aqueous layer was extracted with dichloromethane (2 x 30 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give a brown oil. Purification by flash column chromatography (eluting with 10% methanol in dichloromethane) gave the title compound as a colourless oil (0.48 g).

TLC R_f 0.30 (10% MeOH-CH_2Cl_2)

Intermediate 5 (*RS*)-2-Acetylmercapto-4-methoxycarbonylbutanoic acid

Potassium thiolacetate (2.0 g) was added to a solution of intermediate 1 (3.0 g) in ethanol (30 ml) and the mixture was stirred at RT overnight. The solution was evaporated *in vacuo* and the residue was partitioned between ethyl acetate (30 ml) and water (30 ml). The organic layer was then washed with saturated brine (30 ml), dried ($MgSO_4$) and evaporated *in vacuo* to give the title compound as a colourless oil (1.83 g).

TLC R_f 0.46 (EtOAc)

Similarly prepared were:

Intermediate 6 (*RS*)-2-Acetylmercapto-5-methoxycarbonylpentanoic acid

From Intermediate 2 (1.89 g) as a yellow oil (0.87 g).

TLC R_f 0.30 (5% MeOH-CH_2Cl_2)

Intermediate 7 (*RS*)-2-Acetylmercapto-6-methoxycarbonylhexanoic acid

From Intermediate 3 (4.3 g) as a yellow oil (2.78 g).

TLC R_f 0.23 (5% MeOH-CH_2Cl_2)

Intermediate 8 (*RS*)-(1,1-Dimethylethyl) 2,4-dibromobutyrate

Bromine (31.3ml, 0.54mol) was added dropwise over 4h at 100°C to neat stirring 4-bromobutyryl chloride (100g, 0.54mol). The resulting acid chloride was cooled then added dropwise at 0°Cto a stirred solution of *tert*-butanol (240ml) and triethylamine (64ml, 0,461mol) in anhydrous dichloromethane (600ml). 2M Hydrochloric acid was added and the layers separated. The organic portion was then washed

sequentially with 10% sodium metabisulphite solution (2x500ml), water (500ml) and brine (500ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the title compound (120g, 86%) as a brown liquid.

^{1}H NMR (250MHz; $CDCl_3$), Ref., TMS) 1.50 (9H, s), 2.45 (2H, q), 3.55 (2H, t) and 4.40 (1H, dd).

Similarly prepared were:

Intermediate 9 (*RS*)-(1,1-Dimethylethyl) 2,5-dibromopentanoate

From 5-bromovaleryl chloride (75g, 0.37mol), as a brown oil (81g, 68%).

^{1}H NMR (250MHz; $CDCl_3$), Ref., TMS) 1.50 (9H, s), 1.8-2.3 (4H,m), 3.45 (2H, t) and 4.18 (1H, dd).

Intermediate 10 (*RS*)-(1,1-Dimethylethyl) 2,6-dibromohexanoate

From 6-bromohexanoyl chloride (100g, 0.47mol), as a brown oil (133g, 87%).

^{1}H NMR (250MHz; $CDCl_3$, Ref., TMS) 1.50 (9H, s), 1.6-2.1 (6H, m), 3.4 (2H, t) and 4.1 (1H, dd).

Intermediate 11 (*RS*)-(1,1-Dimethylethyl) 2,4-bis-(acetylmercapto)butyrate

Potassium thiolacetate (1.51g, 13.2mmol)was added to a stirred solution of intermediate 8 (2g, 6.6mmol) in methanol (25ml) and the mixture stirred at RT overnight. The mixture was diluted with dichloromethane (100ml), washed with brine (2x50ml), dried ($MgSO_4$) and evaporated *in vacuo* to a yellow oil. Purification by flash column chromatography (eluting with 30% dichloromethane in hexane) provided the title compound (1.1g, 57%) as a colourless oil.

TLC R_f 0.57 (CH_2Cl_2)

Similarly prepared were:

Intermediate 12 (*RS*)-(1,1-Dimethylethyl) 2,5-bis-(acetylmercapto)pentanoate

From intermediate 9 (2g, 6.32mmol), as a colourless oil (1.12g, 57%)

TLC R_f 0.57 (CH_2Cl_2)

Intermediate 13 (*RS*)-(1,1-Dimethylethyl) 2,6-bis-(acetylmercapto)hexanoate

From intermediate 10 (2g, 6.32mmol), as a colourless oil (1.09g, 57%)

TLC R_f 0.57 (CH_2Cl_2)

Intermediate 14 (*RS*)-2,3-Bis-(acetylmercapto)propionic acid

A solution of thiolacetic acid (1.12 g) in 1N aqueous potassium hydroxide (14.7 ml) was added dropwise to a solution of 2,3-dibromopropionic acid (1.71 g) in 1N

aqueous potassium hydroxide (7.35 ml) and the mixture was stirred 5 h at RT. The pH of the mixture was adjusted to 8-9 by the addition of further 1N aqueous potassium hydroxide and the mixture was stirred a further 2 h, then acidified to pH1-2 by the addition of concentrated hydrochloric acid and extracted with ethyl acetate (2 x 25 ml). The combined extracts were dried (Na_2SO_4) and evaporated to give a yellow oil. The product from two reactions was combined and purification by flash column chromatography (eluting with 4% acetic acid-toluene) gave the title compound as a colourless oil (0.422 g).

TLC R_f 0.15 (5% AcOH-toluene)

Intermediate 15 (*RS*)-2,4-Bis-(acetylmercapto)butyric acid

A solution of intermediate 11 (1.1g, 3.7mmol) in dichloromethane (50ml) was treated with trifluoroacetic acid (2.9ml, 37mmol) and the mixture stirred at RT overnight. Water (50ml) was added and the mixture extracted with dichloromethane (3x40ml). The combined organic extracts were then washed with water (50ml) and brine (50ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the product (870mg, 98%) as a pale yellow oil.

TLC R_f 0.12 (25% MeOH-CH_2Cl_2)

Similarly prepared were:

Intermediate 16 (*RS*)-2,5-Bis-(acetylmercapto)pentanoic acid

From intermediate 12 (1.1g, 3.6mmol), as a pale yellow oil (906mg, 100%)

TLC R_f 0.12 (25% MeOH-CH_2Cl_2)

Intermediate 17 (*RS*)-2,6-Bis-(acetylmercapto)hexanoic acid

From intermediate 13 (1.1g, 3.6mmol), as a pale yellow oil (895mg, 98%)

TLC R_f 0.12 (25% MeOH-CH_2Cl_2)

Intermediate 18 4-Phthalimidobutanoic acid

N-Carboethoxyphthalimide (10.96 g) was added in one portion to a vigorously stirred solution of 4-aminobutanoic acid (5.16 g) and sodium carbonate (5.35 g) in water (150 ml) at RT. The mixture was stirred until essentially all the solid material had dissolved (30 min), then it was filtered. The filtrate was acidified to pH1 with 6N aqueous hydrochloric acid (ca. 22 ml) and the white precipitate was collected by

filtration and washed thoroughly with water (150 ml). The solid was dried in air, then *in vacuo* to give the title compound as a colourless solid (7.35 g).

^{1}H NMR (250 MHz; $CDCl_3$, Ref.,TMS) d 2.03 (2H, pent.) 2.42 (2H, t), 3.78 (2H, t), 7.65-7.77 (2H, m), 7.81-7.90 (2H, m)

Similarly prepared were:

Intermediate 19 5-Phthalimidopentanoic acid

From 5-aminopentanoic acid (5.0 g) as a colourless solid (6.8 g).

^{1}H NMR (250 MHz; $CDCl_3$, Ref., TMS) d 1.6-1.8 (4H, m), 2.20 (2H, t), 3.85 (2H, t), 7.70-7.75 (2H, m), 7.85-7.95 (2H, m), 10.2 (1H, br s)

Intermediate 20 6-Phthalimidohexanoic acid

From 6-aminohexanoic acid (5.0 g) as a colourless solid (5.8 g).

^{1}H NMR (60 MHz; $CDCl_3$, Ref., TMS) 1.5-2.4 (6H, m) 2.3 (2H, t) 3.80 (2H, t), 7.8-8.1 (4H, m), 10.4 (1H, br s)

Intermediate 21 2-(3-Phthalimidophenyl)acetic acid

From 2-(3-aminophenyl)acetic acid (3.0g) as an off-white solid (4.0g, 72%).

TLC R_f 0.36 (7.5% MeOH-0.5% AcOH-CH_2Cl_2)

Intermediate 22 *Cis*-3-Aminocyclopent-4,5-enecarboxylic acid

A solution of racemic lactam (50g, 0.458mol) in 2N hydrochloric acid (1000ml) was heated under reflux for 1h. The mixture was evaporated *in vacuo* and the residue crystallised from acetone to provide the hydrochloride salt of the title compound (73g, 97%) as a white solid.

This hydrochloride salt (20g, 0.122mol)was dissolved in water (300ml) and the stirred solution treated with amberlite (IRA-67) ion exchange resin until pH 7 was reached. The resin was then removed by filtration, the solvent removed *in vacuo* and the residue crystallised from acetone to provide the title compound (13.7g, 88%) as a white solid.

Intermediate 23 *Cis*-3-Phthalimidocyclopent-4,5-enecarboxylic acid

From intermediate 22 hydrochloride (22.3g, 0.136mol), as a white solid (13.7g, 39%).

TLC R_f 0.37 (1% AcOH-5% MeOH-CH_2Cl_2)

Intermediate 24 *Trans*-Methyl 3-Phthalimidocyclopent-4,5-enecarboxylate

Intermediate 23 (23.2g, 0.183mol) and phthalic anhydride (27.03g, 0.183mol) were powdered together and melted at 190°C under nitrogen with stirring. The mixture was allowed to cool to RT and the residue treated with ethyl acetate (120ml). Activated charcoal (1.0g) was added and the mixture heated under reflux for 30min, filtered through celite and the filtrate evaporated *in vacuo* to provide the intermediate phthalimido-acid, a 1:1 mixture of *cis/trans* isomers, as a pale yellow solid (45.7g, 97%).

A solution of this acid in methanol (300ml) was treated with conc. hydrochloric acid (0.5ml) and the mixture heated under reflux for 30min, allowed to cool to RT and the solvent evaporated *in vacuo*. The residue was dissolved in ethyl acetate (400ml) and the solution washed with 8% sodium bicarbonate (2x100ml), water (100ml) and brine (100ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the phthalimido-ester as a 1:1 mixture of *cis/trans* isomers. Separation by flash column chromatography (eluting with 60% ether-pentane) provided the title compound (9.72g, 20%) as a white solid.

TLC R_f 0.46 (40% pentane-ether)

Intermediate 25 *Trans*-3-Phthalimidocyclopent-4,5-enecarboxylic acid

A solution of intermediate 24 (9.72g, 35.8mmol) in a mixture of 0.5N hydrochloric acid (100ml) and glacial acetic acid (100ml) was heated under reflux for 30min. The mixture was diluted with water (200ml) and extracted with ethyl acetate (3x100ml). The combined extracts were washed with brine (100ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the crude product which was crystallised from ether (7.56g, 82%) as a white solid.

TLC R_f 0.48 (1% AcOH-5% MeOH-CH_2Cl_2)

Intermediate 26 *Cis*-3-Phthalimidocyclopentanecarboxylic acid

Intermediate 23 (15.1g, 58.7mmol) was hydrogenated at RT and atmospheric pressure over 5% palladium on charcoal (2g) in ethyl acetate (700ml) overnight. The catalyst was removed by filtration through celite and the filtrate evaporated *in vacuo* to provide the title compound (15g, 98%) as white solid.

TLC R_f 0.37 (1% AcOH-5% MeOH-CH_2Cl_2)

Similarly prepared was:

Intermediate 27 *Trans*-3-Phthalimidocyclopentanecarboxylic acid

From intermediate 25 (7.55g, 29.3mmol), as a white solid (7.04g, 93%).

TLC R_f 0.47 (1% AcOH-5% MeOH-CH_2Cl_2)

Intermediate 28 ***Cis*-(1,1-Dimethylethyl) 3-Phthalimidocyclopentylacetate**

A solution of intermediate 26 (5.09g, 19.6mmol) in dry dichloromethane (60ml) was treated with oxalyl chloride (3.4ml, 39.3mmol) then dimethylformamide (1 drop). The mixture was stirred at RT for 2h then the solvent evaporated *in vacuo* to provide the intermediate acid chloride.

The residue was dissolved in tetrahydrofuran (30ml) then treated with a solution of diazomethane in ether (200ml, *ca.* 80mmol) at 0°C. The mixture was stirred at RT overnight then evaporated *in vacuo* to provide the diazoketone as a yellow solid.

The diazoketone was dissolved in *tert*-butanol (100ml) and heated under reflux while a solution of silver benzoate (438mg, 1.9mmol) in triethylamine (5ml) was added in small portions over 2h. The mixture was heated under reflux for a further 1h then cooled to RT, filtered through celite and the filtrate evaporated *in vacuo* to provide a yellow solid. The residue was dissolved in dichloromethane (75ml) and the solution washed sequentially with 8% sodium bicarbonate (50ml), water (50ml) and brine, dried ($MgSO_4$) and evaporated *in vacuo* to provide the crude product. Purification by flash column chromatography (eluting with 50% ether-pentane) provided the title compound (3.8g, 60%) as a white solid.

TLC R_f 0.52 (50% pentane-ether)

Similarly prepared was:

Intermediate 29 ***Trans*-(1,1-Dimethylethyl) 3-Phthalimidocyclopentylacetate**

From intermediate 27 (1.41g, 5.44mmol), as a white solid (1.22g, 70%).

TLC R_f 0.58 (50% pentane-ether)

Intermediate 30 ***Cis*-3-Phthalimidocyclopentylacetic acid**

A solution of intermediate 28 (1.78g, 5.4mmol) in dichloromethane (20ml) was treated with trifluoroacetic acid (2.1ml, 27mmol) and the mixture stirred at RT overnight. The solvent and excess trifluoroacetic acid was removed *in vacuo* to provide the title compound (1.41g, 96%) as a white solid.

TLC R_f 0.32 (30% pentane-ether)

Similarly prepared was:

Intermediate 31 *Trans*-3-Phthalimidocyclopentylacetic acid

From intermediate 29 (1.18g, 3.58mmol), as a white solid (842mg, 86%).

TLC R_f 0.41 (30% pentane-ether)

Intermediate 32 (*RS*) 2-Bromo-5-phthalimidopentanoic acid

Intermediate 19 (5.0 g 20.2 mmol) and thionyl chloride (10 ml) were heated together at 65°C for 30 min. *N*-Bromosuccinimide (5.4 g) and further thionyl chloride were added, plus 48% aqueous HBr (1 drop). The solution was heated at 60°C for 10 min then 70°C for 2 h 15 min. Further *N*-bromosuccinimide (850 mg) was added and the mixture was heated at 70°C for 2 h. Excess thionyl chloride was removed by evaporation under reduced pressure and the oily residue was diluted with dry tetrahydrofuran (200 ml) and water (200ml). The mixture was then treated cautiously with solid sodium bicarbonate to pH 7-8 then stirred overnight at RT. Excess tetrahydrofuran was removed *in vacuo* and the residue washed with dichloromethane (3x300ml). The aqueous portion was then cautiously acidified to pH 1 using 6M hydrochloric acid and extracted with dichloromethane (4x200ml). The combined extracts were then washed with water (2x400ml) and brine (400ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the product (4.7g, 71%) as a fawn solid.

TLC R_f 0.47 (EtOAc)

Similarly prepared were:

Intermediate 33 (*RS*) 2-Bromo-2-(3-phthalimidophenyl)acetic acid

From intermediate 21 as a colourless solid (588mg, 75%)

TLC R_f 0.19 (5% MeOH-0.1% AcOH-CH_2Cl_2)

Intermediate 34 *Cis*-a-Bromo-3-phthalimidocyclopentylacetic acid

From intermediate 30 (1.41g, 5.16mmol), as a buff foam (1.59g, 87%).

TLC R_f 0.46 (2% MeOH-ether)

Intermediate 35 *Trans*-a-Bromo-3-phthalimidocyclopentylacetic acid

From intermediate 31 (815mg, 2.98mmol), as a yellow-brown foam (805mg, 77%).

TLC R_f 0.48 (2% MeOH-ether)

Intermediate 36 (*RS*) 2-Acetylmercapto-5-phthalimidopentanoic acid

A solution of intermediate 33 (3.0g, 9.2mmol) in methanol (30ml) was treated with potassium thiolacetate (1.05g, 9.2mmol) and the mixture stirred at RT overnight. The mixture was evaporated *in vacuo*, the residue dissolved in dichloromethane

(100ml) then the solution washed with water (2x50ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the product (2.4g, 81%) as a pale yellow foam.

TLC R_f 0.43 (EtOAc)

Similarly prepared were:

Intermediate 37 (*RS*) 2-Acetylmercapto-2-(3-phthalimido)phenylacetic acid

From intermediate 33 as a colourless solid (722mg, 100%)

TLC R_f 0.15 (5% MeOH-0.1% AcOH-CH_2Cl_2)

Intermediate 38 *Cis*-a-(Acetylmercapto)-3-phthalimidocyclopentylacetic acid

From intermediate 34 (2.01g, 5.71mmol), as a brown foam (1.44g, 73%).

TLC R_f 0.42 (Ether)

Intermediate 39 *Trans*-a-(Acetylmercapto)-3-phthalimidocyclopentylaceticacid

From intermediate 35 (774mg, 2.2mmol), as a beige foam (329mg, 43%).

TLC R_f 0.45 (Ether)

Intermediate 40 (*RS*)-*N*-[2-Bromo-4-phthalimidobutanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

Intermediate 18 (2.33 g) and thionyl chloride (2.92 ml) were heated together at 65°C for 30 min. *N*-Bromosuccinimide (2.51 g) and further thionyl chloride were added, plus 48% aqueous HBr (1 drop). The solution was heated at 60°C for 10 min then 70°C for 2 h 15 min. Further *N*-bromosuccinimide (850 mg) was added and the mixture was heated at 70°C for 2 h. Excess thionyl chloride was removed by evaporation under reduced pressure and the oily residue was diluted with dry dichloromethane (10 ml). A portion of the supernatant (4.0 ml) was added to a solution of L-leucyl-L-phenylalanine *N*-methyl amide (500 mg) and triethylamine (0.24 ml) in dry dichloromethane (10 ml) at 0°C, and this mixture was stirred overnight at RT. The mixture was diluted with dichloromethane and washed with saturated aqueous sodium bicarbonate solution (50 ml), 1N aqueous hydrochloric acid (50 ml), and saturated brine (50 ml), then dried ($MgSO_4$) and evaporated *in vacuo* to give a brown solid. This material was purified by flash column chromatography (4 x 18 cm; eluting with 2% methanol-dichloromethane) to give the title compound as an off-white solid (530 mg).

TLC R_f 0.44 (5% MeOH-CH_2Cl_2)

Similarly prepared were:

Intermediate 41 **(*RS*)-*N*-[2-Bromo-5-phthalimidopentanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide**

From Intermediate 19 (4.0 g) and L-leucyl-L-phenylalanine *N*-methyl amide (1.18 g), as a pink solid (1.2 g).

TLC R_f 0.34 (5% MeOH-CH_2Cl_2)

Intermediate 42 **(*RS*)-*N*-[2-Bromo-6-phthalimidohexanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide**

From Intermediate 20 (4.0 g) and L-leucyl-L-phenylalanine *N*-methyl amide (1.18 g), as a near colourless solid (1.0 g).

TLC R_f 0.52 (10% MeOH-CH_2Cl_2)

Intermediate 43 **(*RS*)-2-[(1,1-Dimethylethyl)mercapto]-5-phthalimidopentanoic acid**

Tert-butylthiol (11.3ml, 0.1mol) was added to a stirred solution of potassium *tert*-butoxide (22.45g, 0.1mol) in anhydrous tetrahydrofuran (215ml) and the mixture stirred at RT for 20 min. A solution of intermediate 13 (32.6g, 0.1mol) in anhydrous tetrahydrofuran (80ml) was then added and the mixture stirred at RT overnight. Water (300ml) was added, the mixture acidified to pH 1 with 1N hydrochloric acid and extracted with dichloromethane (3x200ml). The combined extracts were then dried ($MgSO_4$) and evaporated *in vacuo* to provide a yellow oil. Purification by flash column chromatography (eluting with 5-15% dichloromethane in ethyl acetate) then crystallisation from dichloromethane/hexane furnished the title compound

(22g, 66%) as a pale yellow solid.

TLC R_f 0.48 (10% MeOH-CH_2Cl_2)

Intermediate 44 **(*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-phthalimidopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide**

A solution of intermediate 43 (8.06g, 24mmol) and *L*-leucyl-*L*-phenylalanine *N*-methyl amide (7.0g, 24mmol) in dichloromethane (250ml) was treated with *N*-hydroxybenzotriazole (3.9g, 28.9mmol) then EDC (5.06g, 26.4mmol) and the mixture stirred at RT overnight. The mixture was washed with 1N hydrochloric acid (300ml) and the aqueous portion re-extracted with dichloromethane (2x100ml). The

combined extracts were washed sequentially with 1N hydrochloric acid (300ml), 8% sodium bicarbonate (300ml), water (300ml) and brine (300ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide a pale yellow solid. Purification by flash column chromatography (eluting with 5% methanol in dichloromethane) provided the title compound (12.8g, 88%) as a near white solid.

TLC R_f 0.55 (10% MeOH-CH_2Cl_2)

Intermediate 45 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

A solution of intermediate 44 (2.56g, 4.2mmol) in a mixture of tetrahydrofuran (10ml) and ethanol (50ml) was treated with hydrazine hydrate (10ml, xs) and the mixture heated under reflux for 2h. After cooling to RT water (30ml) was added, the solvent removed *in vacuo*, the residue acidified to pH 1 with 1N hydrochloric acid and washed with dichloromethane (2x100ml). The aqueous layer was then basified to pH 14 with 2M sodium hydroxide and extracted with dichloromethane (2x100ml). The combined extracts were washed with brine (100ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide a pale yellow solid. Purification by flash column chromatography (eluting with 10-25% methanol in dichloromethane) provided the title compound (9.2g, 91%) as a near white solid.

TLC R_f 0.23 (30% MeOH-CH_2Cl_2)

Intermediate 46 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(acetylamino)pentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

Acetyl chloride (0.5ml, 7mmol) was added to a stirred solution of intermediate 45 (1.14g, 2.38mmol) and triethylamine (2ml, 14.4mmol) in anhydrous dichloromethane (45ml), the mixture was then stirred at RT overnight. The mixture was diluted with dichloromethane (75ml) and washed successively with 1N hydrochloric acid (100ml), 8% sodium bicarbonate (100ml), water (100ml) and brine (100ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide a pale yellow solid. Purification by flash column chromatography (eluting with 5% methanol in dichloromethane) provided the title compound (1.2g, 95%) as a near white solid.

TLC R_f 0.31 (10% MeOH-CH_2Cl_2)

Similarly prepared were:

Intermediate 47 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(benzoylamino)pentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (2.86g, 5.97mmol) and benzoyl chloride (0.84ml, 7.2mmol), as a near white solid (3.43g, 99%).

TLC R_f 0.41 (10% MeOH-CH_2Cl_2)

Intermediate 48 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-succinimidopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (1.14g, 2.38mmol) and succinic anhydride (0.33g, 3.3mmol), as a near white solid (0.54g, 40%).

TLC R_f 0.58 (10% MeOH-CH_2Cl_2)

Intermediate 49 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(methanesulphonyl) aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (2.03g, 4.24mmol) and methanesulphonyl chloride (0.35ml,4.6mmol), as a near white solid (1.78g, 77%).

TLC R_f 0.46 (10% MeOH-CH_2Cl_2)

Intermediate 50 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(benzenesulphonyl) aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (2.03g, 4.24mmol) and benzenesulphonyl chloride (0.59ml, 4.6mmol), as a near white solid (2.18g, 85%).

TLC R_f 0.54 (10% MeOH-CH_2Cl_2)

Intermediate 51 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(methoxycarbonyl) aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (200mg, 0.43mmol) and methyl chloroformate (0.03ml, 0.41mmol), as a near white solid (196mg, 89%).

TLC R_f 0.32 (5% MeOH-CH_2Cl_2)

Intermediate 52 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(benzyloxycarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (200mg, 0.43mmol) and benzyl chloroformate (0.06ml, 0.41mmol), as a near white solid (230mg, 93%).

TLC R_f 0.44 (5% MeOH-CH_2Cl_2)

Intermediate 53 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(4-pyridylcarbonyl) aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (2.03g, 4.24mmol) and isonicotinoyl chloride (834mg, 4.66mmol), as a near white solid (1.81g, 77%).

TLC R_f 0.22 (10% MeOH-CH_2Cl_2)

Intermediate 54 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(3-pyridylcarbonyl) aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (200mg, 0.42mmol) and nicotinoyl chloride (83mg, 0.47mmol), as a near white solid (130mg, 56%).

TLC R_f 0.26 (10% MeOH-CH_2Cl_2)

Intermediate 55 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(2-pyridylcarbonyl) aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (200mg, 0.42mmol) and picolinoyl chloride (83mg, 0.47mmol), as a near white solid (145mg, 62%).

TLC R_f 0.21 (10% MeOH-CH_2Cl_2)

Intermediate 56 (*RS*)-*N*-[2-[(1,1-Dimethylethyl)mercapto]-5-(2-pyrazinylcarbonyl) aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 45 (200mg, 0.42mmol) and pyrazinoyl chloride (85mg, 0.47mmol), as a near white solid (145mg, 62%).

TLC R_f 0.18 (10% MeOH-CH_2Cl_2)

Intermediate 57 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(acetyl)amino pentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

2-Nitrosulphenyl chloride (3.26g, 6.26mmol) was added to a stirred solution of intermediate 46 (1.23g, 6.49mmol) in glacial acetic acid (75ml) and the mixture stirred at RT overnight. The solvent was removed *in vacuo* and the residue purified by flash column chromatography (eluting with 5% methanol in dichloromethane) to provide the title compound (3.74g, 97%) as a yellow solid.

TLC R_f 0.34 (10% MeOH-CH_2Cl_2)

Similarly prepared were:

Intermediate 58 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(benzoylamino)pentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 47 (1.08g, 5.67mmol), as a yellow solid (3.43g, 99%).
TLC R_f 0.54 (10% MeOH-CH_2Cl_2)

Intermediate 59 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-succinimido pentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 48 (618mg, 1.1mmol), as a yellow solid (665mg, 92%).
TLC R_f 0.25 (10% MeOH-CH_2Cl_2)

Intermediate 60 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(methanesulphonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 49 (1.78g, 3.27mmol), as a yellow solid (1.84g, 88%).
TLC R_f 0.32 (10% MeOH-CH_2Cl_2)

Intermediate 61 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(benzenesulphonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 50 (2.25g, 3.71mmol), as a yellow solid (1.6g, 68%).
TLC R_f 0.37 (10% MeOH-CH_2Cl_2)

Intermediate 62 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(methoxycarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 51 (196mg, 0.31mmol), as a yellow solid (86mg, 38%).
TLC R_f 0.25 (10% MeOH-CH_2Cl_2)

Intermediate 63 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(benzyloxycarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 52 (229mg, 0.32mmol), as a yellow solid (155mg, 60%).
TLC R_f 0.37 (10% MeOH-CH_2Cl_2)

Intermediate 64 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(4-pyridylcarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 53 (1.92g, 3.29mmol), as a yellow solid (1.66g, 74%).

TLC R_f 0.29 (10% MeOH-CH_2Cl_2)

Intermediate 65 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(3-pyridylcarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 54 (190mg, 0.33mmol), as a yellow solid (85mg, 38%).

TLC R_f 0.24 (10% MeOH-CH_2Cl_2)

Intermediate 66 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(2-pyridylcarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 55 (210mg, 0.36mmol), as a yellow solid (120mg, 54%).

TLC R_f 0.21 (10% MeOH-CH_2Cl_2)

Intermediate 67 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-(2-pyrazinylcarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 56 (300mg, 0.51mmol), as a yellow solid (110mg, 42%).

TLC R_f 0.18 (10% MeOH-CH_2Cl_2)

Intermediate 68 (*RS*)-*N*-[2-[(2-Nitrophenylsulphanyl)mercapto]-5-aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

TLC R_f 0.16 (10% MeOH-CH_2Cl_2)

Intermediate 69 (*R*)-*N*-(Phenylmethoxy)carbonyl-(*S*-methyl)-*L*-cysteine

Benzyl chloroformate (11.1ml, 65mmol) was added dropwise to a stirred solution of (*S*-methyl)-*L*-cysteine (10g, 75mmol) in 2M aqueous sodium hydroxide (50ml) and the mixture stirred at RT for 4h. The mixture was then basified to pH14 with further 2M sodium hydroxide and the solution washed with ethyl acetate (4x50ml). The aqueous phase was acidified to pH3 with concentrated hydrochloric acid and then extracted with ethyl acetate (4x70ml). The combined extracts were washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to provide the product as a pale yellow oil (16.8g, 84%).

TLC R_f 0.21 (10% MeOH-$CHCl_3$)

Intermediate 70 (*R*)-*N*-(1,1-Dimethylethoxy)carbonyl-(*S*-methyl)cysteine

Di-tert-butyldicarbonate (8.88g, 40.7mmol) was added to a stirred solution of *S*-methyl-*L*-cysteine (5g, 37mmol) and sodium bicarbonate (7.8g, 92.5mmol) in a mixture of water (150ml) and dioxane (100ml) at 0°C. The mixture was allowed to warm to RT and stirred overnight, then diluted with water (100ml), acidified to pH 3 using 1N hydrochloric acid then extracted with ethyl acetate (3x100ml). The combined extracts were washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to provide the title compound as a colourless oil (6.64g, 76%).

TLC R_f 0.55 (MeOH-H_2O)

Similarly prepared was:

Intermediate 71 (*S*)-*N*-(1,1-Dimethylethoxy)carbonyl-propylglycine

From (*S*)-norvaline (5g, 42.7mmol), as a colourless oil (6.3g, 68%).

TLC R_f 0.50 (MeOH-H_2O)

Intermediate 72 (S)-*N*-(1,1-Dimethylethoxy)carbonyl-(*O*-methyl)serine

Sodium hydride (60% dispersion, 4.3g, 0.107mol) was added portionwise at 0°Cto a stirred solution of (S)-*N*-(1,1-dimethylethoxy)carbonylserine (10g, 48.7mmol) in anhydrous DMF (250ml). A solution of iodomethane (6.1ml, 0.1mol) in anhydrous DMF (10ml) was then added dropwise. The mixture was allowed to warm to RT and stirred overnight, then diluted with 1N hydrochloric acid to pH3 and the solution concentrated *in vacuo* to *ca.* 200ml. The mixture was diluted with water (200ml) and extracted with ethyl acetate (3x100ml). The combined extracts were washed with brine (100ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the title compound as a yellow oil (7.48g, 70%).

TLC R_f 0.39 (10% MeOH-CH_2Cl_2)

Intermediate 73 (*RS*)-*N*-Benzoyl-[b-(4-pyridyl)]alanine methyl ester

a) 2-(4-Pyridyl)-1-(*N*-benzoyl)aminoethylene-1-carboxylic acid hydrochloride

4-pyridylcarboxaldehyde (85g, 0.79mol) was added to a stirred ice cold suspension of sodium acetate (16g,0.195mol) and hippuric acid (150g, 0.84mol) in acetic anhydride (360ml, 3.8mol) an exothermic reaction ensued and the internal temperature reached 30-40°C. The mixture was cooled to RT then poured into water

(1500ml). The resulting solid was removed by filtration washed with water (2x500ml) to provide the intermediate azlactone (74g, 37%) as a brown solid.

The azlactone (70g, 0.28mol) was dissolved in conc. hydrochloric acid (210ml) resulting in the rapid formation of a precipitate which was removed by filtration, washed with acetone (2x500ml) and dried *in vacuo* to provide the product (71g, 78%) as a pale green solid.

b) (*RS*)-*N*-Benzoyl-[b-(4-pyridyl)]alanine hydrochloride

The olefin hydrochloride (70g, 0.23mol) was hydrogenated at RT and atmospheric pressure over 5% palladium on carbon (7g) in water (700ml) overnight. The catalyst was removed by filtration through celite and the solvent concentrated to a volume of *ca.* 100ml. Acetone (1000ml) was added and the resulting precipitate removed by filtration and dried *in vacuo* to provide the product (53g, 75%) as an off-white solid.

c) (*RS*)-*N*-Benzoyl-[b-(4-pyridyl)]alanine methyl ester

Thionyl chloride (15ml, 0.21mol) was added to an ice cold solution of the acid (53g, 0.17mol) in methanol (200ml). The mixture was allowed to warm to RT over 30min before the solvent was removed *in vacuo* providing an off-white semi-solid. The residue was suspended in 8% sodium bicarbonate (500ml) and the product extracted into ethyl acetate (4x400ml). The combined organic extracts were then washed with brine (1000ml), dried ($MgSO_4$) and evaporated *in vacuo* providing the title compound (46g, 94%) as an off-white solid.

$C_{16}H_{16}N_2O_3$ [284.3]; [MH^+ 285]

Similarly prepared were:

Intermediate 74 (*RS*)-*N*-Benzoyl-[b-(3-pyridyl)]alanine methyl ester

In three steps from pyridine-3-carboxaldehyde as a white solid.

$C_{16}H_{16}N_2O_3$ [284.3]; [MH^+ 285]

Intermediate 75 (*RS*)-*N*-Benzoyl-2-methoxyalanine methyl ester

In three steps from 2-methoxybenzaldehyde as a white solid

$C_{17}H_{19}N_2O_4$ [301.3]; [MH^+302]

Intermediate 76 (*S*)-*N*-(1,1-Dimethylethoxy)carbonyl-[b-(4-pyridyl)]alanine

Intermediate 73 (27g, 0.104mol) was dissolved in hot acetone (50ml) and the solution added to 0.03M potassium dihydrogen phosphate (500ml) at pH7.2.

Alcalase (1ml) was then added to the vigorously stirred solution and the pH maintained at 7.2 by the addition of 1M sodium hydroxide (*ca.* 20ml) over 30 min. Undissolved ester was filtered off and the filtrate washed with ethyl acetate (4x250ml). The solvent was then evaporated *in vacuo*, the residue dissolved in 6N hydrochloric acid (100ml) then heated under reflux for 5h. After cooling to RT the precipitated benzoic acid was removed by filtration and the filtrate washed with ethyl acetate (2x100ml). The aqueous portion was then evaporated *in vacuo* to provide the optically pure amino-acid as its dihydrochloride salt (9.48g, 81%).

The dihydrochloride salt was dissolved in water (150ml) and treated with Amberlite IRA-67 ion exchange resin to pH 8. The resin was removed by filtration through celite and the solution evaporated *in vacuo* to provide the free amino-acid as a white solid (6.7g, 100%).

Di-*tert*-butyldicarbonate (13g, 60mmol) was added to a stirred suspension of the amino-acid in a mixture of *tert*-butanol (100ml) and water (50ml) while the pH was maintained at 8.5 by the addition of 1M sodium hydroxide (55ml) over 40min. Water (50ml) was added, the mixture washed with heptane (2x100ml) then acidified to pH 3.5 by the addition of solid potassium hydrogen sulphate. The mixture was then evaporated to dryness and the residue triturated with methanol (3x150ml) to extract the product. Evaporation of the solvent *in vacuo* provided the title compound as a white solid which was recrystallised from aqueous ethanol to optical purity (3.27g, 61%).

$[a]_D$ $+24.3^0$ (c=1, Trifluoroacetic acid)

Similarly prepared were:

Intermediate 77 (*S*)-(1,1-Dimethylethoxy)carbonyl-[b-(3-pyridyl)]alanine

From intermediate 74 as a white solid

$[a]_D$ $+16.1^0$ (c=1, Trifluoroacetic acid)

Intermediate 78 (*S*)-(1,1-Dimethylethoxy)carbonyl-2-methoxyphenylalanine

From intermediate 75 as a white solid (11.1g,97%)

$[a]_D$ -15.2^0 (c=1, MeOH)

Intermediate 79 (*RS*)-*N*-Acetyl-[b-(2-pyridyl)]alanine

A solution of sodium methoxide (65.8g, 1.22mol) in methanol (300ml) was treated portionwise with diethylacetamidomalonate (132.3g, 0.61mol) maintaining a

temperature of *ca.* 45°C. The mixture was then heated under reflux for 15min. The mixture was cooled to 50°C then treated slowly with a suspension of 2-chloromethylpyridine hydrochloride (100g, 0.61mol), the pink suspension was then heated under reflux for a further 6h. Water (500ml) was added followed by 10M sodium hydroxide (122ml, 1.22mol) and the pH was maintained at *ca.* 11 while heating the mixture at 70°C overnight. The mixture was cooled to RT and the methanol removed *in vacuo*. The aqueous residue was washed with ethyl acetate (2x500ml), then acidified to pH 5 and further washed with ethyl acetate (2x500ml), before evaporating *in vacuo* to provide a semi-solid. The residue was triturated with hot ethanol (500ml) and the sodium chloride removed by filtration. The filtrate was then evaporated *in vacuo* and the residue crystallised from methanol-ethyl acetate to provide the title compound (70g, 66%) as a yellow solid.

TLC R_f 0.5 [*n*-BuOH-AcOH-pyridine-H_2O (15-3-10-12)]

Similarly prepared were:

Intermediate 80 (*RS*)-*N*-Acetyl-[b-(4-thiazolyl)]alanine

From 4-(chloromethyl)thiazole, as a white foam (6.7g, 73%).

TLC Rf 0.37 (1% AcOH-20% Hexane-EtOAc)

Intermediate 81 (*S*)-(1,1-Dimethylethoxy)carbonyl-[b-(2-pyridyl)]alanine

A suspension of intermediate 79 (65g, 0.31mol) in aqueous potassium dihydrogen orthophosphate (10mM, 650ml) was warmed to 40°C and the resulting mixture (pH 4) basified to pH 8 with 10M sodium hydroxide (10ml) forming a solution. Acylase 30,000 (1.3g) was added and the mixture incubated at 40°C overnight. The resulting suspension was cooled to RT, the solid removed by filtration, then the filtrate acidified to pH 1 with 2N hydrochloric acid and washed with ethyl acetate (2x300ml). The aqueous portion was evaporated *in vacuo* to provide a semi-solid which was triturated with hot methanol (300ml) and the solid removed by filtration. To the cooled solution was then added 5M sodium hydroxide to pH 10, followed by di-*tert*-butyldicarbonate (28.6g, 0.131mol), the mixture was maintained at pH 10 by the addition of 5M sodium hydroxide (44ml) over a period of 6h. The methanol was then removed *in vacuo*, the aqueous residue washed with ether (2x500ml) then acidified to pH 3 with 1M potassium hydrogen sulphate. The mixture was extracted

with ethyl acetate (4x500ml), the combined extracts washed with brine (500ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the crude product which was crystallised from ethyl acetate as a white solid (11.6g, 23%).

$[a]_D$ -16.0° (c=1, MeOH)

Similarly prepared were:

Intermediate 82 (*S*)-*N*-(1,1-Dimethylethoxy)carbonyl-[b-(4-thiazolyl)]alanine

From intermediate 80, as a white solid (11g, 94%).

HPLC (Chirex (D)-penicillamine; 2mM $CuSO_4$; 0.7ml/min; RT=3.25min; 99%ee.

Intermediate 83 (*S*)-*N*-(1,1-Dimethylethoxy)carbonyl[b-(2-pyridyl)]alanine *N*-methyl amide

DCC (4.07g, 19.7mmol) was added to an ice cooled solution of intermediate 81 (5.0g, 18.8mmol) and *N*-hydroxysuccinimide (2.27g, 19.7mmol) in dry tetrahydrofuran (200ml). After stirring at RT for 3h the mixture was treated with 40%w/v aqueous methylamine (6.8ml, 79 mmol) and stirred for a further 2h. The precipitated solid was removed by filtration, the filtrate evaporated *in vacuo* and the residue dissolved in dichloromethane (100 ml). The solution was washed sequentially with water (2x100ml), 8% sodium bicarbonate (2x100ml) and brine (100ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the crude product.

Purification by column chromatography eluting with 2-5% methanol/dichloromethane provided the title compound (2.7g, 51%) as a red foam.

TLC R_f 0.38 (3% MeOH-CH_2Cl_2)

Similarly prepared were:

Intermediate 84 (*S*)-*N*-(1,1-Dimethylethoxy)carbonyl-L-[b-(4-thiazolyl)]alanine *N*-methyl amide

From intermediate 82, as a white solid (3.0g, 64%).

TLC R_f 0.46 (5% MeOH-CH_2Cl_2)

Intermediate 85 (*R*)-*N*-(1,1-Dimethylethoxy)carbonylpenicillamine *N*-methyl amide

A solution of (*R*)-*N*-(1,1-dimethylethoxy)carbonylpenicillamine (14g, 56.1mmol), *N*-hydroxybenzotriazole (7.6g, 56.1mmol), methylamine hydrochloride (18.9g, 280mmol), *N*-methylmorpholine (34ml, 308mmol), 4-dimethylaminopyridine (685mg, 5.6mmol) and EDC (11.8g, 62mmol) in anhydrous dimethylformamide

(300ml) was stirred at RT overnight. The mixture was poured into 10%w/v citric acid (750ml) and extracted with ether (3x500ml). The combined extracts were then washed with 8% sodium bicarbonate (2x500ml) and brine (500ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the title compound (12.3g, 83%)

TLC R_f 0.47 (50% hexane-EtOAc)

Similarly prepared was:

Intermediate 86 (1,1-Dimethylethoxy)carbonyl-5-methyl-L-glutamic acid *N*-methyl amide

From (1,1-dimethylethoxy)carbonyl-5-methyl-L-glutamic acid, as a white solid (5.4g, 94%).

TLC R_f 0.21 (2% MeOH-CH_2Cl_2)

Intermediate 87 (*S*)-N_d-(1,1-Dimethylethoxy)carbonyl-N_a-(benzyloxy)carbonylornithine *N*-methyl amide

From (*S*)-N_d-(1,1-dimethylethoxy)carbonyl-N_a-(benzyloxy)carbonylornithine (11.5g, 31.4mmol), as a white solid (11.0g, 94%).

TLC R_f 0.65 (3% MeOH-CH_2Cl_2)

Intermediate 88 (*S*)-*N*-(1,1-Dimethylethoxy)carbonyl-2-methoxyphenylalanine *N*-methyl amide

From intermediate 78 (500mg, 1.69mmol), as a white solid (380mg, 73%).

TLC R_f 0.55 (5% MeOH-CH_2Cl_2)

Intermediate 89 (*S*)-*N*-(1,1-Dimethylethoxy)carbonyl-b-(3-pyridyl)alanine *N*-methyl amide

From intermediate 77 (2.0g, 7.5mmol), as a white solid (1.0g, 48%).

TLC R_f 0.42 (5% MeOH-CH_2Cl_2)

Intermediate 90 (*S*)-*N*-(1,1-Dimethylethoxy)carbonyl-b-(4-pyridyl)alanine *N*-methyl amide

From intermediate 76 (2.0g, 7.5mmol), as a white solid (1.7g, 81%).

TLC R_f 0.38 (5% MeOH-CH_2Cl_2)

Intermediate 91 (*R*)-*N*-(1,1-Dimethylethoxy)carbonyl-(*S*-methyl)penicillamine *N*-methyl amide

A solution of iodomethane (0.59ml, 9.55mmol) in methanol (5ml) was added dropwise at 0°C to a stirred solution of intermediate 85 (500mg, 1.51mmol) in a

mixture of 2M sodium hydroxide (5ml) and methanol (15ml). The mixture was stirred at RT overnight, then the methanol removed *in vacuo*, the residue diluted with water (50ml) and extracted with ether (3x50ml). The combined extracts were washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to provide the title compound as a colourless oil (540mg, 99%).

TLC R_f 0.47 (50%hexane-EtOAc)

Intermediate 92 **(*R*)-*N*-(1,1-Dimethylethoxy)carbonyl-2,2-dimethyl-2-methane sulphonylalanine *N*-methyl amide**

A suspension of Oxone (1.02g, 1.66mmol) in water (5ml) was added to a stirred solution of intermediate 91 (153mg, 0.55mmol) in methanol (5ml) at 0°C, the mixture was allowed to warm to RT and stirred for 4h. The mixture was diluted with water (50ml) and extracted with ethyl acetate (3x50ml). The combined extracts were washed with brine (50ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the title compound as a colourless oil (101mg, 59%).

TLC R_f 0.35 (50%hexane-EtOAc)

Intermediate 93 **(*R*)-*N*-(1,1-Dimethylethoxy)carbonyl-2,2-dimethyl-2-methane sulphinylalanine *N*-methyl amide**

A solution of 3-chloroperbenzoic acid (670mg, 2.89mmol) in dichloromethane (25ml) was added to a stirred solution of intermediate 91 (800mg, 2.89mmol) in dichloromethane (25ml) at 0°C, the mixture was allowed to warm to RT and stirred overnight. The mixture was washed sequentially with 10%w/v sodium sulphite (2x50ml), 8% sodium bicarbonate (2x50ml) and brine (50ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the crude product as a colourless oil. Purification by flash column chromatography (eluting with 10% methanol-dichloromethane) provided the title compound as a colourless oil (470mg, 55%).

TLC R_f 0.05 (50%hexane-EtOAc)

Intermediate 94 **(*R*)-2,2-Dimethyl-2-methanesulphinylalanine *N*-methyl amide hydrochloride**

A solution of intermediate 93 (470mg, 1.61mmol) in a mixture of dioxane (25ml) and 2M hydrochloric acid (25ml) was stirred at RT overnight. The solvent was

evaporated to dryness *in vacuo* and freeze dried overnight to provide the title compound (370mg, 100%) as a colourless gum.

TLC R_f 0.06 (1% NEt_3-10% MeOH-CH_2Cl_2)

Similarly prepared were:

Intermediate 95 (*R*)-2,2-Dimethyl-2-methanesulphonylalanine *N*-methyl amide hydrochloride

From intermediate 92 (300mg, 0.97mmol), as a colourless foam (210mg, 100%).

TLC R_f 0.10 (1% NEt_3-10% MeOH-CH_2Cl_2)

Intermediate 96 (*R*)-Penicillamine *N*-methyl amide hydrochloride

From intermediate 85 (300mg, 1.14mmol), as a colourless foam (230mg, 100%).

TLC R_f 0.13 (1% NEt_3-10% MeOH-CH_2Cl_2)

Intermediate 97 (*S*)-b-(3-Pyridyl)alanine *N*-methyl amide dihydrochloride

From intermediate 89 (1.2g, 4.29mmol), as a white solid (1.1g, 100%).

TLC R_f 0.05 (1% NEt_3-10% MeOH-CH_2Cl_2)

Intermediate 98 (*S*)-b-(4-Pyridyl)alanine *N*-methyl amide dihydrochloride

From intermediate 90 (1.6g, 5.73mmol), as a white solid (1.45g, 100%).

TLC R_f 0.08 (1% NEt_3-10% MeOH-CH_2Cl_2)

Intermediate 99 (*S*)-b-(2-Pyridyl)alanine *N*-methyl amide dihydrochloride

From intermediate 83 (1.4g, 5.01mmol), as a white solid (1.27g, 100%).

TLC R_f 0.13 (1% NEt_3-10% MeOH-CH_2Cl_2)

Intermediate 100 (*S*)-b-(4-Thiazolyl)alanine *N*-methyl amide hydrochloride

From intermediate 84 (1.0g, 3.5mmol), as a white solid (730mg, 94%).

TLC R_f 0.21 (1% NEt_3-10% MeOH-CH_2Cl_2)

Intermediate 101 (*R*)-(*S*-Methyl)penicillamine *N*-methyl amide trifluoroacetate

A solution of intermediate 91 (200mg, 0.72mmol) in dichloromethane (4ml) containing trifluoroacetic acid (2ml) was stirred at RT overnight. The solvent was removed *in vacuo* and any excess trifluoroacetic acid removed by azeotropic distillation with heptane (3x20ml) to provide the title compound (196mg, 94%) as a colourless foam.

TLC R_f 0.32 (1% NEt_3-10% MeOH-CH_2Cl_2)

Similarly prepared were:

Intermediate 102 (*S*)-2-(Methoxyphenyl)alanine *N*-methyl amide trifluoroacetate

From intermediate 88 (200mg, 0.65mmol), as a pale yellow foam (209mg, 100%).

TLC R_f 0.25 (1% NEt_3-10% MeOH-CH_2Cl_2)

Intermediate 103 (*S*)-5-Methyl-glutamic acid *N*-methyl amide trifluoroacetate

From intermediate 86 (707mg, 2.58mmol), as a white solid (728mg, 98%).

TLC R_f 0.37 (1% NEt_3-10% MeOH-CH_2Cl_2)

Intermediate 104 (*S*)-N_δ-(1,1-Dimethylethoxy)carbonylornithine *N*-methyl amide

Intermediate 87 (11g, 29mmol) was hydrogenated at RT and atmospheric pressure over 10% palladium on carbon (1g) in ethanol overnight. The catalyst was removed by filtration through hyflo and the filtrate evaporated *in vacuo* to provide the title compound (2.6g, 36%) as a colourless oil.

TLC R_f 0.37 (5% MeOH-CH_2Cl_2)

Intermediate 105 ***N*-(Phenylmethoxy)carbonyl-(*S*-methyl)-*L*-cysteinyl-*L*-phenylalanine *N*-methyl amide**

EDC (10.5g, 55mmol) was added to a stirred solution of *L*-phenylalanine *N*-methyl amide (8.9g, 50mmol), intermediate 69 (13.8g, 50mmol) and *N*-hydroxybenzotriazole (8.1g, 60mmol) in dry tetrahydrofuran (100ml). The mixture was stirred at RT overnight. The mixture was treated with 1M hydrochloric acid (300ml) then extracted with ethyl acetate (4x200ml). The combined organic extracts were washed with 8% sodium bicarbonate (2x200ml), water (200ml) and brine (200ml), dried ($MgSO_4$) and evaporating *in vacuo* to provide the product as a white solid (16.5g, 75%).

TLC R_f 0.47 (10% MeOH-$CHCl_3$)

Intermediate 106 ***N*-(1,1-Dimethylethoxy)carbonyl-(*S*)-propylglycinyl-*L*-phenylalanine *N*-methyl amide**

From intermediate 71 (5g, 23mmol) and *L*-phenylalanine *N*-methyl amide (4.1g, 23mmol), as a white solid (7.72g, 89%).

TLC R_f 0.48 (10% MeOH-CH_2Cl_2)

Intermediate 107 ***N*-(1,1-Dimethylethoxy)carbonyl-*L*-leucyl-*L*-*tert*-leucine *N*-methyl amide**

From *N*-(1,1-dimethylethoxy)carbonyl-*L*-leucine (13.07g, 56mmol) and *L*-*tert*-leucine *N*-methyl amide (8.11g, 56mmol), as a white solid (15.77g, 79%).

TLC R_f 0.25 (5% MeOH-CH_2Cl_2)

Intermediate 108 *N*-(1,1-Dimethylethoxy)carbonyl-(*S*)-(*S*-methyl)cysteinyl-*L*-*tert*-leucine *N*-methyl amide

From intermediate 70 (6.64g, 28mmol) and *L*-*tert*-leucine *N*-methyl amide (4.07g, 28mmol), as a white solid (4.26g, 42%).

TLC R_f 0.45 (10% MeOH-CH_2Cl_2)

Intermediate 109 *N*-(1,1-Dimethylethoxy)carbonyl-(*S*)-(*O*-methyl)serinyl-*L*-*tert*-leucine *N*-methyl amide

From intermediate 72 (7.48g, 34mmol) and *L*-*tert*-leucine *N*-methyl amide (4.92g, 34mmol), as a white solid (4.26g, 42%).

TLC R_f 0.40 (10% MeOH-CH_2Cl_2)

Intermediate 110 *N*-(Phenylmethoxy)carbonyl-*L*-valinyl-N_δ-(1,1-dimethylethoxy) carbonyl-*L*-ornithine *N*-methyl amide

From intermediate 104 (2.6g, 10.6mmol) and *N*-(Phenylmethoxy)carbonyl-*L*-valine (2.82g, 11.2mmol), as a white solid (3.0g, 61%).

TLC R_f 0.32 (5% MeOH-CH_2Cl_2)

Intermediate 111 (*S*-Methyl)-*L*-cysteinyl-*L*-phenylalanine *N*-methyl amide

A solution of intermediate 105 (2.0g, 4.65mmol) in dichloromethane (10ml) was treated with 25% hydrobromic acid in acetic acid (18.6ml) and the mixture stirred at RT for 1h. Water (10ml) was added and the mixture washed with dichloromethane (3x15ml). The aqueous phase was then basified to pH14 with 5M sodium hydroxide then extracted with dichloromethane (4x30ml). The combined organic extracts were then washed with brine (30ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the product as a white solid (1.22g, 88%).

TLC R_f 0.31 (10% MeOH-$CHCl_3$)

Intermediate 112 *L*-Valinyl-N_δ-(1,1-dimethylethoxy)carbonyl-*L*-ornithine *N*-methylamide

Intermediate 110 (3.0g, 6.5mmol) was hydrogenated at RT and atmospheric pressure over 10% palladium on carbon (300mg) in ethanol (100ml) overnight. The catalyst

was removed by filtration through hyflo and the filtrate evaporated *in vacuo* to provide the title compound (2.2g, 99%) as a white solid.

TLC R_f 0.26 (10% MeOH-CH_2Cl_2)

Intermediate 113 (*S*)-Propylglycinyl-*L*-phenylalanine *N*-methyl amide

A solution of intermediate 106 (5.36g, 14.2mmol) in a mixture of dioxane (250ml) and 2M hydrochloric acid (250ml) was stirred at RT overnight. The solvent was evaporated to dryness *in vacuo* to provide a white solid. The residue was dissolved in water (200ml) washed with dichloromethane (3x100ml) then basified to pH 10 with 2M sodium hydroxide and extracted with dichloromethane (4x100ml). The combined extracts were washed with brine (100ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the title compound as a white solid (1.45g, 37%).

TLC R_f 0.29 (10% MeOH-CH_2Cl_2)

Similarly prepared were:

Intermediate 114 (*S*)-(*S*-Methyl)cysteinyl-*L-tert*-leucine *N*-methyl amide

From intermediate 108, as a white solid (3.5g, 97%).

TLC R_f 0.45 (10% MeOH-CH_2Cl_2)

Intermediate 115 (*S*)-(*O*-Methyl)serinyl-*L-tert*-leucine *N*-methyl amide

From intermediate 109, as a white solid (2.7g, 98%).

TLC R_f 0.40 (10% MeOH-CH_2Cl_2)

Intermediate 116 *L-Leucyl-L-tert*-leucine *N*-methyl amide

From intermediate 107, as a white solid (11.2g, 99%).

TLC R_f 0.57 (10% MeOH-CH_2Cl_2)

Intermediate 117 (*R*)-2-Bromo-5-phthalimidopentanoic acid

A solution of *D*-ornithine hydrochloride (35g, 0.208mol) in water (350ml) was treated with copper (II) sulphate (16.6g, 0.104mol). 5M Potassium hydroxide (*ca.* 40ml) was added to pH 3 then *N*-carboethoxyphthalimide (45.5g, 0.208mol) was added and the pH maintained at 9-10 by the addition of 5M potassium hydroxide (*ca.*55ml). After 2h, 48% hydrobromic acid was added to pH 0.4 (*ca.* 77ml) and any resulting precipitate removed by filtration. The filtrate was cooled to $< 5^0$C, then treated with further hydrobromic acid (152ml) and potassium bromide (59g, 0.5mol). The mixture was then treated dropwise over 45 min with a solution of sodium nitrite

(28.6g, 0.41mol) in water (275ml) whilst maintaining a temperature of $<5^0$C. The mixture was then stirred at $<5^0$C overnight. The resulting precipitate was then removed by filtration, dissolved in ethyl acetate (400ml) and the solution washed with water (2x200ml) and brine (200ml), dried ($MgSO_4$) and evaporated *in vacuo* to provide the title compound (40.6g, 47%) as a cream solid.

TLC R_f 0.80 (10% MeOH-CH_2Cl_2)

Similarly prepared was:

Intermediate 118 (*S*)-2-Bromo-5-phthalimidopentanoic acid

From L-ornithine hydrochloride (20g, 0.118mol), as a cream solid (22g, 57%).

TLC R_f 0.80 (10% MeOH-CH_2Cl_2)

Intermediate 119 (*S*)-2-Acetylmercapto-5-phthalimidopentanoic acid

Was prepared by the previously described procedure from intermediate 117 (39.9g, 0.11mol), as a pale orange oil (35.6g, 99%).

TLC R_f 0.24 (50% Heptane-EtOAc)

Intermediate 120 (*R*)-2-Acetylmercapto-5-phthalimidopentanoic acid

Was prepared by the previously described procedure from intermediate 118 (20g, 56mmol), as a pale orange oil (17.7g, 99%).

TLC R_f 0.24 (50% Heptane-EtOAc)

Intermediate 121 (*RS*)- 2-(Acetylmercapto)-5-phthalimidopentanoyl-L-leucine 1,1-dimethylethyl ester

EDC (3.64g, 19mmol) was added to a stirred mixture of L-leucine 1,1-dimethylethyl ester (3.93g, 17.6mmol), *N*-hydroxybenzotriazole (2.62g, 19.4mmol), triethylamine (2.51ml, 18mmol) and intermediate 15 (5.94g, 18.5mmol) in dry tetrahydrofuran (200ml). The mixture was stirred overnight then the solvent removed *in vacuo* and the residue partitioned between water (100ml) and ethyl acetate (100ml). The aqueous portion was then extracted with ethyl acetate (2x50ml), the combined extracts washed with water (2x100ml) and brine (100ml), dried ($MgSO_4$) and evaporated *in vacuo* to a colourless oil.

Purification by column chromatography eluting with hexane/ethyl acetate (2:1) provided the title compound (6.6g, 77%) as a white solid, a 1:1 mixture of diastereoisomers.

TLC R_f 0.42 (EtOAc/Hexane (1:1))

Intermediate 122 (*RS*)-2-(Acetylmercapto)-5-phthalimidopentanoyl-L-leucine

Trifluoroacetic acid (9.0ml, 115mmol) was added to a stirred solution of intermediate 121 (3.0g, 6.1mmol) in dry dichloromethane (40ml) and the mixture stirred at RT overnight. The mixture was concentrated *in vacuo* and the excess trifluoroacetic acid removed by azeotroping with heptane to provide the title compound (2.48g, 94%) as a colourless foam, a 1:1 mixture of diastereoisomers.

TLC R_f 0.42 (EtOAc/Hexane (3:2))

Intermediate 123 (*RS*)-2-(Acetylmercapto)-4-succinimidobutanoic acid

Was prepared by the procedure previously described for intermediate 36

TLC R_f 0.38 (HOAc/EtOAc/Hexane (0.1:1:1))

Intermediate 124 (*S*-Methyl)-L-cysteinyl-L-tryptophan *N*-methyl amide

Was prepared by the procedure previously described for intermediate 111

TLC R_f 0.45 (EtOAc/Hexane (1:1))

Intermediate 125 (*RS*)-2-[(1,1-Dimethylethyl)mercapto]-3-phthalimidopropananoic acid

Was prepared by the procedure previously described for intermediate 43.

TLC R_f 0.48 (HOAc/EtOAc/Hexane (0.1:1:1))

Example 1 (*RS*)-*N*-[2,3-Bis-acetylmercaptopropanoyl]-L-leucyl-L-phenylalanine *N* methyl amide

EDC (198 mg) was added to a solution of intermediate 14 (209 mg), L-leucyl-L-phenylalanine *N*-methyl amide (274 mg), and *N*-hydroxybenzotriazole hydrate (153 mg) in dry THF (10 ml) at 0°C, and the mixture was stirred at that temperature until TLC analysis (5% MeOH-CH_2Cl_2) indicated a complete consumption of starting materials (72 h). The solvent was removed by evaporation and the residue was partitioned between 1N hydrochloric acid (35 ml) and ethyl acetate (50 ml). The organic layer was separated, washed with aqueous sodium bicarbonate solution (2 x 200 ml) and brine (2 x 20 ml), then dried ($MgSO_4$) and evaporated *in vacuo* to give the crude product. Purification by flash column chromatography (eluting with

2-5% methanol-dichloromethane) gave the title compound as a colourless solid (264 mg).

TLC R_f 0.25 (2% MeOH-CH_2Cl_2)

Similarly prepared were:

Example 2 (*RS*)-*N*-[(2,4-Bis-acetylmercapto)butanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 15 (870mg, 3.7mmol) and *L*-leucyl-*L*-phenylalanine *N*-methyl amide (1.07g, 3.7mmol), as a white solid (1.4g, 74%).

$C_{24}H_{35}N_3O_5S_2$ [509.7]; [MH^+=510]

Example 3 (*RS*)-*N*-[(2,5-Bis-acetylmercapto)pentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 16 (906mg, 3.6mmol) and *L*-leucyl-*L*-phenylalanine *N*-methyl amide (1.06g, 3.6mmol), as a white solid (1.5g, 79%).

$C_{25}H_{37}N_3O_5S_2$ [523.7]; [MH^+=524]

Example 4 (*RS*)-*N*-[(2,6-Bis-acetylmercapto)hexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 17 (895mg, 3.4mmol) and *L*-leucyl-*L*-phenylalanine *N*-methyl amide (991mg, 3.4mmol), as a white solid (1.4g, 74%).

$C_{26}H_{39}N_3O_5S_2$ [537.75]; [MH^+=538]

Example 5 (*RS*)-*N*-[2-Acetylmercapto-3-methoxycarbonylpropranoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From Intermediate 4 (0.26 g), as a colourless solid (0.33 g).

$C_{23}H_{33}N_3O_6S$ [479.6]; [MH^+=480]

Example 6 (*RS*)-*N*-[2-Acetylmercapto-4-methoxycarbonylbutanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From Intermediate 5 (0.40 g), as a colourless solid (0.67 g).

$C_{24}H_{35}N_3O_6S$ [493.6]; [MH^+=494]

Example 7 (*RS*)-*N*-[2-Acetylmercapto-5-methoxycarbonylpentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From Intermediate 6 (0.9 g), as a colourless solid (1.1 g).

$C_{25}H_{37}N_3O_6S$ [507.6]; [MH^+=508]

Example 8 (*RS*)-*N*-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From Intermediate 7 (0.95 g), as a colourless solid (1.0 g).

$C_{26}H_{39}N_3O_6S$ [521.6]; [MH^+=522]

Example 9 (*RS*)-*N*-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-tryptophan *N*-methyl amide

From Intermediate 36 (730mg) and *L*-leucyl-*L*-tryptophan *N*-methyl amide (700mg), as a pale yellow foam (1.0g, 74%)

$C_{33}H_{39}N_5O_6S$ [633.7]; [MH^+=634]

Example 10 (*RS*)-*N*-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-tryptophan *N*-methyl amide

From Intermediate 7 (375mg) and *L*-leucyl-*L*-tryptophan *N*-methyl amide (500mg), as a pale yellow foam (440mg, 52%)

$C_{28}H_{40}N_4O_6S$ [560.7]; [MH^+=561]

Example 11 (*RS*)-*N*-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-valinyl-*L*-phenylalanine *N*-methyl amide

From Intermediate 36 (232mg) and *L*-valinyl-*L*-phenylalanine *N*-methyl amide (200mg), as a white solid (230mg, 55%)

$C_{30}H_{36}N_4O_6S$ [580.7]; [MH^+=581]

Example 12 (*RS*)-*N*-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-valinyl-*L*-phenylalanine *N*-methyl amide

From Intermediate 7 (179mg) and *L*-valinyl-*L*-phenylalanine *N*-methyl amide (200mg), as a white solid (200mg, 56%)

$C_{25}H_{37}N_3O_6S$ [507.6]; [MH^+=508]

Example 13 (*RS*)-*N*-[2-Acetylmercapto-2-(3-phthalimidophenyl)acetyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From Intermediate 37 (690mg) and *L*-leucyl-*L*-phenylalanine *N*-methyl amide (489mg), as a white solid (927mg, 88%)

$C_{34}H_{36}N_4O_6S$ [628.8]; [MH^+=629]

Example 14 *N*-[2-(Acetylmercapto)-2-[3-*cis*-phthalimidocyclopentyl]acetyl-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 38 (140mg, 0.4mmol) and *L*-leucyl-*L*-phenylalanine *N*-methyl amide (117mg, 0.4mmol), as a tan foam (197mg, 79%), a 1:1:1:1 of the four expected diastereoisomers.

$C_{33}H_{40}N_4O_6S$ [620.8]; [MH^+=621]

Example 15 ***N*-[2-(Acetylmercapto)-2-[3-*trans*-phthalimidocyclopentyl]acetyl-*L*-leucyl-*L*-phenylalanine *N*-methyl amide**

From intermediate 39 (235mg, 0.7mmol) and *L*-leucyl-*L*-phenylalanine *N*-methyl amide (197mg, 0.7mmol), as a brown foam (358mg, 85%), a 1:1:1:1 of the four expected diastereoisomers.

$C_{33}H_{40}N_4O_6S$ [620.8]; [MH^+=621]

Example 16 **(*S*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(S)-*tert*-leucine *N*-methyl amide**

From intermediate 119 (13.8g, 43mmol) and intermediate 116 (11.2g, 44mmol), as a white solid (11.6g, 48%).

$C_{28}H_{40}N_4O_6S$ [560.7]; [MH^+=561]

Example 17 **(*S*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-phenylalanine *N*-methyl amide**

From intermediate 119 (10g, 31mmol) and L-leucyl-L-phenylalanine *N*-methyl amide (9.0g, 31mmol), as a white solid (10.5g, 57%).

$C_{31}H_{38}N_4O_6S$ [594.7]; [MH^+=595]

Example 18 **(*R*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-phenylalanine *N*-methyl amide**

From intermediate 120 (1.0g, 3.1mmol) and L-leucyl-L-phenylalanine *N*-methyl amide (900mg, 3.1mmol), as a white solid (885mg, 48%).

$C_{31}H_{38}N_4O_6S$ [594.7]; [MH^+=595]

Example 19 **(*S*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-tryptophan *N*-methyl amide**

From intermediate 119 (411mg, 1.28mmol) and L-leucyl-L-tryptophan *N*-methyl amide (423mg, 1.28mmol), as a white solid (330mg, 41%).

$C_{33}H_{39}N_5O_6S$ [633.7]; [MH^+=634]

Example 20 *(RS)-N*-[2-(Acetylmercapto)-5-phthalimidopentanoyl]-(*S*-methyl)-*L*-cysteinyl-*L*-phenylalanine *N*-methyl amide

From intermediate 36 (326mg, 1mmol) and intermediate 114 (350mg, 1mmol), as a white solid (380mg, 64%).

$C_{29}H_{34}N_4O_6S_2$ [598.7]; [MH^+=599]

Example 21 *(RS)-N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-(S)-propylglycinyl-L-phenylalanine *N*-methyl amide

From intermediate 113 (145mg, 0.52mmol) and intermediate 36 (168mg, 0.52mmol), as a white solid (160mg, 53%).

$C_{30}H_{36}N_4O_6S$ [580.7]; [MH^+=581]

Example 22 *(RS)-N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-valinyl-N_δ-(1,1-dimethylethoxy)carbonyl-L-ornithine *N*-methyl amide

From intermediate 112 (508mg, 1.47mmol) and intermediate 36 (491mg, 1.53mmol), as a white solid (687mg, 72%).

$C_{31}H_{45}N_5O_8S$ [647.8]; [MH^+=648]

Example 23 *(RS)-N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-valinyl-L-ornithine *N*-methyl amide trifluoroacetate

A solution of example 22 (585mg, 0.90mmol) in dichloromethane (20ml) containing trifluoroacetic acid (2ml) was stirred at RT overnight. The solvent was removed *in vacuo* and any excess trifluoroacetic acid removed by azeotropic distillation with heptane (3x20ml) to provide the title compound (596mg, 99%) as a off white solid.

$C_{26}H_{38}N_5O_6S$ [548.7]; [MH^+=549]

Example 24 *(RS)-N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-valinyl-N_δ-(acetyl-L-ornithine *N*-methyl amide

Acetyl chloride (0.024ml, 0.34mmol) was added at 0°C to a stirred solution of example 23 (205mg, 0.31mmol) and *N*-methylmorpholine (0.134ml, 0.31mmol) in anhydrous dichloromethane (20ml). The mixture was allowed to warm to RT and stirred for 1h before diluting with dichloromethane (20ml) and washing sequentially with 2N hydrochloride (20ml), 8% sodium bicarbonate (20ml), water (20ml) and brine (20ml), dried ($MgSO_4$) and evaporated *in vacuo* to a colourless foam.

Purification by flash column chromatography (eluting with 3-5% methanol-dichloromethane) provided the title compound (30mg, 17%) as a white solid.

$C_{28}H_{41}N_5O_7S$ [590.7]; [MH^+=591]

Example 25 (*RS*)-*N*-[2-Acetylmercapto-4-phthalimidobutanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

A solution of potassium thiolacetate (98 mg) in methanol (2 ml) was added to a suspension of Intermediate 40 (0.5 g) in methanol (10 ml) and the mixture was stirred at RT for 30 min, then at reflux for 6h. The solvent was then removed under reduced pressure and the residue was partitioned between water (50 ml) and dichloromethane (150 ml). The layers were separated and the organic layer was dried over sodium sulfate, filtered, and evaporated to give the crude product. Purification by flash column chromatography (eluting with 50% ethyl acetate-dichloromethane) gave the title compound as a colourless solid (270 mg).

TLC R_f 0.30 (50% EtOAc-CH_2Cl_2)

Similarly prepared were:

Example 26 (*RS*)-*N*-[2-Acetylmercapto-5-phthalimidopentanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

From Intermediate 41 (0.50 g) as a near colourless solid (0.42 g).

TLC R_f 0.20 (50% EtOAc-CH_2Cl_2)

Example 27 (*RS*)-*N*-[2-Acetylthio-6-phthalimidohexanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

From Intermediate 42 (0.50 g), as a pale yellow solid (0.39 g).

TLC R_f 0.31 (50% EtOAc-CH_2Cl_2)

Example 28 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-[β-(4-thiazolyl)]alanine *N*-methyl amide

EDC (107mg, 0.56mmol) was added to a stirred solution of intermediate 122 (222mg,0.51 mmol), *N*-hydroxybenzotriazole 76mg, 0.56mmol), triethylamine (75ml, 0.53mmol) and intermediate 100 (113mg, 0.51mmol) in dry tetrahydrofuran (30ml). The mixture was stirred at RT overnight then the solvent removed *in vacuo* and the residue partitioned between water (20ml) and ethyl acetate (20ml). The aqueous portion was then extracted with ethyl acetate (2x20ml), the combined

extracts washed with water (2x50ml) and brine (50ml), dried ($MgSO_4$) and evaporated *in vacuo* to a pale yellow oil.

Purification by column chromatography eluting with dichloromethane/methanol (98:2) provided the title compound (170mg, 55%) as a white solid.

$C_{28}H_{35}N_5O_6S_2$ [601.7]; [MH^+=602]

Similarly prepared were:

Example 29 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-[β-(2-pyridyl)]alanine *N*-methyl amide

From intermediate 122 and intermediate 99, as a white solid (277mg, 67%).

$C_{30}H_{37}N_5O_6S$ [595.7]; [MH^+=596]

Example 30 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-[β-(3-pyridyl)]alanine *N*-methyl amide

From intermediate 122 and intermediate 97, as a white solid (50mg, 12%).

$C_{30}H_{37}N_5O_6S$ [595.7]; [MH^+=596]

Example 31 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-L-[β-(4-pyridyl)]alanine *N*-methyl amide

From intermediate 122 and intermediate 98, as a white solid (310mg, 77%).

$C_{30}H_{37}N_5O_6S$ [595.7]; [MH^+=596]

Example 32 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-5-methyl-L-glutamic acid *N*-methyl amide

From intermediate 122 and intermediate 103, as a white solid (201mg, 49%).

$C_{28}H_{38}N_4O_8S$ [590.3]; [MH^+=591]

Example 33 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(*S*)-(2-methoxyphenyl)alanine *N*-methyl amide

From intermediate 122 and intermediate 102, as a white solid (150mg, 37%).

$C_{32}H_{40}N_4O_7S$ [624.8]; [MH^+=625]

Example 34 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(*R*)-penicillamine *N*-methyl amide

From intermediate 122 and intermediate 96, as a white solid (230mg, 35%).

$C_{27}H_{38}N_4O_6S_2$ [578.8]; [MH^+=579]

Example 35 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(*R*)-(*S*-methyl)-penicillamine *N*-methyl amide

From intermediate 122 and intermediate 101, as a white solid (400mg, 35%).

$C_{28}H_{40}N_4O_6S_2$ [592.8]; [MH^+=593]

Example 36 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(*R*)-(2-dimethyl-2-methanesulphonyl)alanine *N*-methyl amide

From intermediate 122 and intermediate 95, as a white solid (380mg, 66%).

$C_{28}H_{40}N_4O_8S_2$ [624.8]; [MH^+=625]

Example 37 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(*R*)-(2-dimethyl-2-methanesulphinyl)alanine *N*-methyl amide

From intermediate 122 and intermediate 94, as a white solid (360mg, 37%).

$C_{28}H_{40}N_4O_7S_2$ [608.8]; [MH^+=609]

Example 38 (*RS*)-*N*-[2-(Acetylmercapto)-5-phthalimido]pentanoyl-L-leucyl-(*S*)-*tert*-leucine *N*-methyl amide

From intermediate 122 and *tert*-leucine *N*-methyl amide hydrochloride, as a white solid (120mg, 25%).

$C_{28}H_{40}N_4O_6S$ [560.7]; [MH^+=561]

Example 39 (*RS*)-*N*-[2-Mercapto-5-(acetyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

0.4M Sodium hydroxide (0.82ml) was added to a stirred solution of intermediate 57 (203mg, 0.328mmol) and 2-mercaptoethanol (0.23ml, 3.28mmol) in methanol at 0°C. After 15min acetic acid (0.5ml) was added and the solvents evaporated *in vacuo* to provide a yellow oil. Ether (20ml) was added and the resulting precipitate removed by filtration to provide the crude thiol. Purification by flash column chromatography (eluting with 5% methanol in dichloromethane) provided the title compound (86mg, 56%) as a white solid.

$C_{23}H_{36}N_4O_4S$ [464.6]; [MH^+=465]

Similarly prepared were:

Example 40 (*RS*)-*N*-[2-Mercapto-5-(benzoyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 58 (400mg, 0.59mmol), as a white solid (175mg, 59%).

$C_{28}H_{38}N_4O_4S$ [526.7]; [MH^+=527]

Example 41 (*RS*)-*N*-[2-Mercapto-5-(succinimido)pentanoyl]-*L*-l ucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 59 (600mg, 0.91mmol), as a white solid (147mg, 32%).

$C_{25}H_{36}N_4O_5S$ [504.7]; [MH^+=505]

Example 42 (*RS*)-*N*-[2-Mercapto-5-(methanesulphonyl)aminopentanoyl]-*L*-leucyl-*L* phenylalanine *N*-methyl amide

From intermediate 60 (600mg, 1.0mmol), as a white solid (262mg, 57%).

$C_{22}H_{36}N_4O_5S_2$ [500.7]; [MH^+=501]

Example 43 (*RS*)-*N*-[2-Mercapto-5-(benzenesulphonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 61 (500mg, 0.7mmol), as a white solid (221mg, 56%).

$C_{27}H_{36}N_4O_5S_2$ [562.8]; [MH^+=563]

Example 44 (*RS*)-*N*-[2-Mercapto-5-(4-pyridylcarbonyl)aminopentanoyl]-*L*-leucyl-*L* phenylalanine *N*-methyl amide

From intermediate 64 (330mg, 0.48mmol), as a white solid (115mg, 22%).

$C_{27}H_{37}N_5O_4S$ [527.7]; [MH^+=528]

Example 45 (*RS*)-*N*-[2-Mercapto-5-(3-pyridylcarbonyl)aminopentanoyl]-*L*-leucyl-*L* phenylalanine *N*-methyl amide

From intermediate 65 (200mg, 0.29mmol), as a white solid (34mg, 22%).

$C_{27}H_{37}N_5O_4S$ [527.7]; [MH^+=528]

Example 46 (*RS*)-*N*-[2-Mercapto-5-(2-pyridylcarbonyl)aminopentanoyl]-*L*-leucyl-*L* phenylalanine *N*-methyl amide

From intermediate 66 (150mg, 0.22mmol), as a white solid (60mg, 52%).

$C_{27}H_{37}N_5O_4S$ [527.7]; [MH^+=528]

Example 47 (*RS*)-*N*-[2-Mercapto-5-(2-pyrazinylcarbonyl)aminopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methyl amide

From intermediate 67 (100mg, 0.15mmol), as a white solid (30mg, 38%).

$C_{26}H_{36}N_6O_4S$ [528.7]; [MH^+=529]

Example 48 (*RS*)-*N*-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-L-[β-(4-thiazolyl)]alanine *N*-methyl amide

Concentrated ammonium hydroxide (0.5ml) was added to a solution of example 28 (110mg, 0.18mmol) in methanol (10ml) at 0°C and the mixture was stirred at that temperature for 3 h. The mixture was diluted with water (10 ml), acidified with 2N aqueous hydrochloric acid and extracted with dichloromethane (3x20ml). The

combined extracts were dried (Na_2SO_4) filtered and evaporated to give the crude product. Purification by flash column chromatography (eluting with 2% methanol-dichloromethane) gave the title compound as a colourless solid (71mg, 71%).

$C_{26}H_{33}N_5O_5S_2$ [559.7]; [MH^+=560]

Similarly prepared were:

Example 49 (*RS*)-*N*-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-L-[β-(2-pyridyl)]alanine *N*-methyl amide

From example 29, as a white solid (75mg, 80%).

$C_{28}H_{35}N_5O_5S$ [553.7]; [MH^+=554]

Example 50 (*RS*)-*N*-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-5-methyl-L-glutamic acid *N*-methyl amide

From example 32, as a white solid (68mg, 78%).

$C_{26}H_{36}N_4O_7S$ [548.6]; [MH^+=549]

Example 51 (*RS*)-*N*-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-(*R*)-(*S*-methyl)-penicillamine *N*-methyl amide

From example 35, as a white solid (160mg, 70%).

$C_{26}H_{38}N_4O_5S_2$ [550.8]; [MH^+=551]

Example 52 (*S*)-*N*-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-(*S*)-*tert*-leucine *N*-methyl amide

From example 16, as a white solid (8.12g, 80%).

$C_{26}H_{38}N_4O_5S$ [518.7]; [MH^+=519]

Example 53 (*S*)-*N*-[2-Mercapto-5-phthalimido]pentanoyl-L-leucyl-L-phenylalanine *N*-methyl amide

From example 17, as a white solid (2.7g, 67%).

$C_{29}H_{36}N_4O_5S$ [552.7]; [MH^+=553]

Example 54 (*RS*)-*N*-[2,3-Dimercaptopropanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

From example 1, as a colourless solid (76 mg).

$C_{19}H_{29}N_3O_3S_2$ [411.5]; [MH^+=412]

Example 55 (*RS*)-*N*-[2-Mercapto-3-methoxycarbonylpropanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

From example 5 (0.16 g), as a colourless solid (0.14 g).

$C_{21}H_{31}N_3O_5S_2$ [437.5]; [MH^+=438]

Example 56 (*RS*)-*N*-[2-Mercapto-4-methoxycarbonylbutanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

From example 6 (0.18 g), as a colourless solid (0.16 g).

$C_{22}H_{33}N_3O_5S$ [451.5]; [MH^+=452]

Example 57 (*RS*)-*N*-[2-Mercapto-5-methoxycarbonylpentanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

From example 7 (0.32 g), as a colourless solid (0.15 g).

TLC R_f 0.29 (5% MeOH-CH_2Cl_2)

Example 58 (*RS*)-*N*-[2-Mercapto-6-methoxycarbonylhexanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

From example 8 (0.31 g), as a colourless solid (0.22 g).

TLC R_f 0.30 (5% MeOH-CH_2Cl_2)

Example 59 (*RS*)-*N*-[2-Mercapto-4-phthalimidobutanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

From example 25 (0.20 g), as a pale yellow solid (0.12 g).

$C_{28}H_{34}N_4O_5S$ [538.6]; [MH_2^+=540]

Example 60 (*RS*)-*N*-[2-Mercapto-5-phthalimidopentanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

From example 26 (0.2 g), a a colourless solid (0.18 g).

TLC R_f 0.41 (5% MeOH-CH_2Cl_2)

Example 61 (*RS*)-*N*-[2-Mercapto-6-phthalimidohexanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

From example 27 (0.15 g), as a pale yellow solid (0.12 g).

TLC R_f 0.25 (10% MeOH-CH_2Cl_2)

Example 62 (*RS*)-*N*-[2-Mercapto-5-phthalimidopentanoyl]-L-leucyl-L-tryptophan*N*-methyl amide

From example 9 (250mg), as a pale yellow foam (222mg, 96%).

$C_{31}H_{37}N_5O_5S$ [591.7]; [MH^+=592]

Example 63 (*RS*)-*N*-[2-Mercapto-6-methoxycarbonylhexanoyl]-L-leucyl-L-tryptophan *N*-methyl amide

From example 10 (330mg), as a colourless foam (300mg, 98%).

$C_{26}H_{38}N_4O_5S$ [518.7]; [MH^+ =519]

Example 64 (*S*)-*N*-[2-Mercapto-5-phthalimidopentanoyl]-L-(*S*-methyl)cysteinyl-L-tryptophan *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 119 and intermediate 124.

$C_{29}H_{32}N_5O_5S_2$ [595.7]; [MH^+ =596]

Example 65 (*RS*)-*N*-[2-Mercapto-3-phthalimidopropanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

Was prepared by the procedure described previously for example 39 *via* intermediate 43, from intermediate 125 and L-leucyl-L-phenylalanine *N*-methyl amide.

$C_{27}H_{32}N_4O_5S$ [524.6]; [MH^+ =525]

Example 66 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and L-leucyl-L-phenylalanine *N*-methyl amide.

$C_{24}H_{34}N_4O_5S$ [490.6]; [MH^+ =491]

Example 67 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-(*S*)-propylglycinyl-L-phenylalanine *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 113.

$C_{23}H_{31}N_4O_5S$ [475.6]; [MH^+ =476]

Example 68 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-L-(*S*-methyl)cysteinyl-L-*tert*-leucine *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 114.

$C_{19}H_{32}N_4O_5S_2$ [460.6]; [MH^+ =461]

Example 69 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-L-(*S*-methyl)cysteinyl-L-tryptophan *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 124.

$C_{24}H_{31}N_5O_5S_2$ [533.7]; [MH^+=534]

Example 70 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-L-(*S*-methyl)cysteinyl-L-phenylalanine *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 111.

$C_{23}H_{30}N_4O_5S_2$ [506.6]; [MH^+=507]

Example 71 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-L-(*O*-methyl)serinyl-L-*tert*-leucine *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 115.

$C_{19}H_{32}N_4O_5S$ [444.5]; [MH^+=445]

Example 72 (*RS*)-*N*-[2-Mercapto-4-succinimidobutanoyl]-L-leucyl-L-*tert*-leucine *N*-methyl amide

Was prepared by the procedure described previously for examples 28 and 48, from intermediate 123 and intermediate 116.

$C_{21}H_{36}N_4O_5S$ [456.6]; [MH^+=457]

Example 73 (*RS*)-*N*-[2-Mercapto-6-carboxyhexanoyl]-L-leucyl-L-tryptophan *N*-methyl amide

Was prepared by hydrolysis of example 10.

$C_{25}H_{36}N_4O_5S$ [504.7]; [MH^+=505]

Example 74 (*R*)-*N*-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-L-leucyl-L-tryptophan *N*-methyl amide

Was prepared by separation of the 1:1 mixture of diastereoisomers present in example 10 by flash column chromatography.

$C_{26}H_{38}N_4O_5S$ [518.7]; [MH^+=519]

Example 75 (*S*)-*N*-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-L-leucyl-L-tryptophan *N*-methyl amide

Was prepared by separation of the 1:1 mixture of diastereoisomers present in example 10 by flash column chromatography.

$C_{26}H_{38}N_4O_5S$ [518.7]; [MH^+=519]

Example 76 (*R*)-*N*-[2-Acetylmercapto-5-phthalimidopentanoyl]-L-leucyl-L-tryptophan *N*-methyl amide

Was prepared by separation of the 1:1 mixture of diastereoisomers present in example 9 by flash column chromatography.

$C_{31}H_{37}N_5O_5S_2$[591.7]; [MH^+=592]

Example 77 (*R*)-*N*-[2-Acetylmercapto-5-methoxycarbonylpentanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

Was prepared by separation of the 1:1 mixture of diastereoisomers present in example 7 by flash column chromatography.

$C_{25}H_{36}N_4O_5S$ [504.7]; [MH^+=505]

Example 78 (*RS*)-*N*-[2-Mercapto-6-(methylamino)carbonylhexanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

Was prepared by hydrolysis of example 8.

$C_{24}H_{38}N_4O_4S$ [478.6]; [MH^+=479]

Example 79 (*RS*)-*N*-[2-Mercapto-6-(amino)carbonylhexanoyl]-L-leucyl-L-phenylalanine *N*-methyl amide

Was prepared by hydrolysis of example 8.

$C_{23}H_{36}N_4O_4S$ [464.6]; [MH^+=465]

Example A

Collagenase inhibition activity

The potency of compounds of general formula (I) to act as inhibitors of collagenase was determined by the procedure of Cawston and Barrett, (Anal. Biochem., 99:340-345, 1979) whereby a 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with collagen and collagenase (buffered with 50 mM Tris, pH 7.6 containing 5 mM $CaCl_2$, 0.05% Brij 35, 60 mM NaCl and 0.02% NaN_3). The collagen was acetylated ^3H or ^{14}C-collagen prepared by the method of Cawston and Murphy (Methods in Enzymolgy, 80:711, 1981). The choice of radiolabel did not alter the ability of collagenase to degrade the collagen substrate. The samples were centrifuged to sediment undigested collagen and an aliquot of the radioactive supernatant removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1mM inhibitor, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the collagenase (IC_{50}).

Example B

Stromelysin inhibition activity

The potency of compounds of general formula (I) to act as inhibitors of stromelysin was determined using the procedure of Nagase et al (Methods in Enzymology Vol 254, 1994), whereby a 0.1 mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with stromelysin and ^3H transferrin (buffered with 50 mM Tris, pH 7.6 containing 10 mM $CaCl_2$, 150M NaCl, 0.05% Brij, 35, and 0.02% NaN_3). The transferrin was carboxymethylated with ^3H iodoacetic acid. The stromelysin activity in the presence of 1 mM, or a dilution thereof, was compared to activity in a control devoid of inhibitor and the results reported as that inhibitor concentration effecting 50% inhibition of the stromelysin (IC_{50})

Example C

Gelatinase inhibition activity

The potency of the compounds of general formula (I) to act as inhibitors of gelatinase was determined using the procedure of Harris & Krane (Biochem Biophys. Acta, 258:566 - 576, 1972), whereby a 1 mM solution of the inhibitor being tested or dilutions thereof was incubated at 37°C for 16 hours with gelatinase and heat denatured ^3H or ^{14}C-acetylated collagen (buffered with 50 mM Tris, pH 7.6 containing 5 mM $CaCl_2$, 0.05% Brij 35 and 0.02% NaN_3). The ^3H or ^{14}C gelatin was prepared by denaturing ^3H or ^{14}C-collagen produced according to the method of Cawston and Murphy (Methods in Enzymology, 80:711, 1981) by incubation at 60°C for 30 minutes. Undigested gelatin was precipitated by addition of trichloroacetic acid and centrifugation. The gelatinase activity in the presence of 1 mM, or dilution thereof, was compared to the activity in a control devoid of inhibitor and results reported as that inhibitor concentration effecting 50% inhibition of the gelatinase (IC_{50}).

Example D

Inhibition of TNFα production

The potency of the compounds of general formula (I) to act as inhibitors of the production of TNFa was determined using the following procedure. A 1mM solution of the inhibitor being tested or dilutions thereof was incubated at 37° C in an

atmosphere of 5% CO_2 with THP-1 cells (human monocytes) suspended in RPMI 1640 medium and 20μM β-mercaptoethanol at a cell density of 1 x 10^6/ml and stimulated with 5μg/ml final concentration of LPS. After 18 hours the supernatant is assayed for the levels of TNFα using a commercially available ELISA kit (R & D Systems).

The activity in the presence of 0.1mM inhibitor or dilutions thereof was compared to activity in a control devoid of inhibitor and results reported as that inhibitor concentration effecting 50% inhibition of the production of TNFa.

Example E

Adjuvant arthritic rat model

Compounds of general formula (I) were evaluated in an adjuvant arthritis model in the rat based on the methods employed by B.B. Newbould (1963), Br.J.Pharmacol, 21, 127-136 and C.M. Pearson and F.D. Wood (1959), Arthritis Rheum, 2, 440-459. Briefly male Wistar rats (180-200g) were injected at the base of the tail with Freund's adjuvant. Twelve days later the responding animals were randomised into experimental groups. Compounds of general formula (I) were dosed either orally as a suspension in 1% methyl cellulose or intraperitoneally in 0.2% carboxymethylcellulose from day 12 to the end of the experiment on day 22. Hind paw volumes were measured every two days from day 12 onwards and X-rays were taken of the hind feet on completion of the experiment. Results were expressed as the percent increase of foot volume over day 12 values.

Example F

Mouse ovarian carcinoma xenograft model

Compounds of general formula (I) were evaluated in an ovarian carcinoma xenograft model of cancer, based on that described by B. Davies et al (1993), Cancer Research, 53, 2087-2091 This model, in brief, consists of inoculating female nu/nu mice with 1 x 10^9 OVCAR3-icr cells into the peritoneal cavity. Compounds of general formula (I) are administered by the oral route as a suspension in 1% methyl cellulose or intraperitoneally as a suspension in phosphate buffered saline in 0.01% Tween-20. At the conclusion of the experiment (4-5 weeks) the number of peritoneal cells are counted and any solid tumour deposits weighed. In some

experiments tumour development is monitored by measurement of tumour specific antigens.

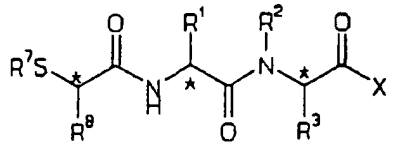
Example G

Rat mammary carcinoma model

Compounds of general formula (I) were evaluated in a HOSP.1 rat mammary carcinoma model of cancer (S.Eccles et al (1995), Cancer Research, in press). This model consists of the intravenous inoculation of female CBH/cbi rats with 2 x 10^4 tumour cells into the jugular vein. Compounds of general formula (I) are administered by the oral route as a suspension in 1% methyl cellulose or intraperitoneally as a suspension in phosphate buffered saline in 0.01% Tween-20. At the conclusion of the experiment (4-5 weeks) the animals were killed, the lungs were removed and individual tumours counted after 20 hours fixation in Methacarn.

CLAIMS

1. A compound of general formula (I):



(I)

wherein:

R^1 is C_{1-6} alkyl, C_{2-6} alkenyl, -C_{1-6} alkyl-aryl, aryl, -C_{1-6} alkyl-heteroaryl, heteroaryl or -C_{1-6} alkyl-AR^9 where A represents O,NR^9 or $S(O)_m$ where m=0-2, and R^9 is H, C_{1-4} alkyl, aryl, heteroaryl, -C_{1-4} alkyl-aryl or -C_{1-4} alkyl-heteroaryl; if $A=NR^9$ the groups R^9 may be the same or different;

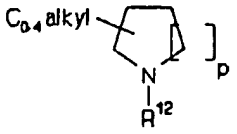
R^2 is H or C_{1-6} alkyl;

R^3 is $[Alk]_nR^6$ where Alk is C_{1-6} alkyl or C_{2-6} alkenyl and n is zero or 1;

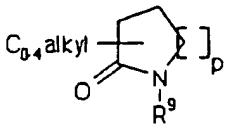
X is NR^4R^5 where either R^4 is hydrogen or C_{1-6} alkyl optionally substituted by amino (NH_2), aryl, arylamino, protected amino, di(C_{1-6} alkyl)amino, mono(C_{1-6} alkyl)amino, CO_2H, protected carboxyl, carbamoyl, mono(C_{1-6} alkyl)carbamoyl or di(C_{1-6} alkyl)carbamoyl, and R^5 is hydrogen or C_{1-6} alkyl; or NR^4R^5 forms a ring such as pyrrolidino, piperidino or morpholino;

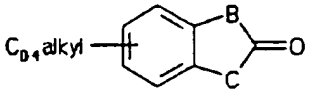
R^7 is hydrogen or $R^{10}CO$ where R^{10} is C_{1-4} alkyl, -C_{1-4} alkyl-aryl, -C_{1-4} alkyl-heteroaryl, cyclo(C_{3-6})alkyl, -C_{1-4} alkyl-cyclo(C_{3-6})alkyl, C_{2-6} alkenyl, -C_{2-6} alkenyl-aryl, aryl or heteroaryl;

R^8 is aryl (substituted with R^{11}), heteroaryl (optionally substituted with R^{11}), C_{1-4} alkyl-R^{11}, -C_{1-4} alkyl-aryl (substituted with R^{11}), -C_{1-4} alkyl-heteroaryl (optionally substituted with R^{11}), cyclo(C_{3-6})alkyl (optionally substituted with R^{11}), cyclo(C_{3-6})alkenyl (optionally substituted with R^{11}), -C_{1-4} alkyl-cyclo(C_{3-6})alkyl (optionally substituted with R^{11}), or any of the three groups



or





where p is 1 or 2 and B and C are independently selected from O, S, $C(R^9)_2$ and NR^9;

R^6 is AR^9, cyclo(C_{3-6})alkyl, cyclo(C_{3-6})alkenyl, C_{1-6} alkyl, -C_{1-6} alkoxy-aryl, benzyloxyaryl, aryl, heteroaryl, -C_{1-3} alkyl-heteroaryl, -C_{1-3} alkyl-aryl, -C_{1-6} alkyl-$COOR^9$, -C_{1-6} alkyl-NHR, CONHR, $NHCO_2R$, $NHSO_2R$ or NHCOR, R being defined as for R^{10};

R^{11} is SO_2R^{13}, SR^7, SR^9, COR^{13}, $N(R^9)_2$, NR^9R^{12}, OR^9, succinimido or phthalimido;

R^{12} is H or COR^9, CO_2R^9 (where R^9 is not H), $CONHR^9$ or SO_2R^9 (where R^9 is not H); and

R^{13} is OH, OC_{1-4} alkyl, O-C_{1-4} alkyl-aryl, $N(R^9)_2$ (in which the R^9s are the same or different), C_{1-4} alkyl, aryl, heteroaryl, -C_{1-4} alkyl-aryl or -C_{1-4} alkyl-heteroaryl;

the compound being in the form of a non-salt, salt, solvate or hydrate.

2. A compound of claim 1, wherein R^1 is C_{1-6} alkyl or C_{1-6} alkyl-AR^9 where A is $S(O)_m$, NR^9 or O and m=0, 1 or 2, and R^9 is H, C_{1-4} alkyl or aryl.

3. A compound of claim 1 or claim 2, wherein R^3 is $[Alk]_nR^6$ where n=0 or 1, Alk is C_{1-6} alkyl and R^6 is C_{1-6} alkyl, -C_{1-3} alkyl-aryl, -C_{1-3} alkyl-heteroaryl or AR^9.

4. A compound of any preceding claim, wherein R^4 is H.

5. A compound of any preceding claim, wherein X is pyrrolidino, piperidino or morpholino.

6. A compound of any preceding claim, wherein R^7 is H or (C_{1-6} alkyl)carbonyl.

7. A compound of any preceding claim, wherein R^8 is C_{1-4} alkyl-R^{11} or cyclo(C_{3-6})alkyl-R^{11}, and R^{11} is COR^{13}, NR^9R^{12}, $N(R^9)_2$, succinimido or phthalimido, R^{12} is COR^9, CO_2R^9 (provided R^9 is not H) or SO_2R^9 (provided R^9 is not H), and R^{13} is OH, OC_{1-4} alkyl or $N(R^9)_2$.

8. A compound of any preceding claim, wherein R^5 is H or C_{1-6} alkyl.

9. A compound of claim 8, wherein:

R^1 is alkyl, alkenyl, alkylaryl, aryl or alkyl-AR^9 and R^9 is alkyl, aryl or heteroaryl;

R^7 is H or $R^{10}CO$ where R^{10} is alkyl, alkylaryl, cycloalkyl, cycloalkylalkyl, alkenyl or alkenylaryl;

R^8 is optionally-substituted aryl, heteroaryl, alkylaryl, cycloalkyl, cycloalkenyl or alkylcycloalkyl, alkyl-R^{11} or any of the said three groups;

R^6 is cycloalkyl, cycloalkenyl, alkyl, benzyl, alkoxybenzyl, benzyloxylbenzyl or 3-indolylmethyl;

R^{11} is SR^7, SR^9, COR^{13}, $N(R^9)_2$, NR^9R^{12}, OR^9, succinimido or phthalimido; and

R^{13} is OH, Oalkyl, Oalkylaryl, $N(R^9)_2$, alkyl, aryl or alkylaryl.

10. A compound of claim 1, selected from

N-[2,3-*bis*-Acetylmercaptopropanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-3-methoxycarbonylpropanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-4-methoxycarbonylbutanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-5-methoxycarbonylpentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-4-phthalimidobutanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-6-phthalimidohexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2,3-*bis*-Mercaptopropanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-3-methoxycarbonylpropanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-4-methoxycarbonylbutanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-5-methoxycarbonylpentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-4-phthalimidobutanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide and

N-[2-Mercapto-6-phthalimidohexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide.

11. A compound of claim 1, selected from

N-[2-Acetylmercapto-5-methoxycarbonylpentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-valinyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-tryptophan *N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-valinyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-tryptophan *N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-[β-(4-thiazolyl)]alanine*N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-[β-(2-pyridyl)]alanine *N*-methylamide

N-[2-Acetylmercapto-5-phthalimidopentanoyl]-*L*-leucyl-5-methyl-*L*-glutamicacid *N*-methylamide

N-[2-Acetylmercapto-6-phthalimidohexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Acetylmercapto-2-(3-phthalimido)phenylacetyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-5-methoxycarbonylpentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-6-methoxycarbonylhexanoyl]-*L*-leucyl-*L*-tryptophan *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-tryptophan *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-[β-(4-thiazolyl)]alanine *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-*L*-[β-(2-pyridyl)]alanine *N*-methylamide

N-[2-Mercapto-5-phthalimidopentanoyl]-*L*-leucyl-5-methyl-*L*-glutamic acid *N*-methylamide and

N-[2-Mercapto-6-phthalimidohexanoyl]-*L*-leucyl-*L*-phenylalanine *N*-methylamide.

12. A compound of any of claims 1 to 9, wherein R^1 is $-CH_2SCH_3$.

13. A compound of claim 12, as defined in any of Examples 20, 64, 68, 69 and 70.

14. A compound of any of claims 1 to 9, wherein R^3 is tert-butyl or $-C(CH_3)_2S(O)_{0-2}CH_3$.

15. A compound of claim 14, as defined in any of Examples 16, 34, 35, 36, 37, 38, 51, 52 and 72.

16. A compound of any preceding claim, in the form of a single enantiomer or diastereomer, or a mixture of such isomers.

17. A pharmaceutical composition for use in therapy, comprising a compound of any preceding claim, and a pharmaceutically-acceptable diluent or carrier.

18. Use of a compound of any of claims 1 to 16, for the manufacture of a medicament for the treatment or prevention of a condition associated with matrix metalloproteinases or that is mediated by TNFα.

19. Use according to claim 18, wherein the condition is selected from cancer, inflammation and inflammatory diseases, tissue degeneration, periodontal disease, ophthalmological disease, dermatological disorders, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia and anorexia, acute infection, HIV infection, shock states, graft versus host reactions, autoimmune disease, reperfusion injury, meningitis and migraine.

20. Use according to claim 19, wherein the condition is selected from tumour growth, angiogenesis, tumour invasion and spread, metastases, malignant ascites and malignant pleural effusion.

21. Use according to claim 19, wherein the condition is selected from rheumatoid arthritis, osteoarthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's disease, atherosclerosis, stroke, vasculitis, Crohn's disease and ulcerative colitis.

22. Use according to claim 19, wherein the condition is selected from corneal ulceration, retinopathy and surgical wound healing.

23. Use according to claim 19, wherein the condition is selected from psoriasis, atopic dermatitis, chronic ulcers and epidermolysis bullosa.

24. Use according to claim 19, wherein the condition is periodonititis or gingivitis.

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/GB 95/02362

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/06 A61K38/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,95 13289 (CHIROSCIENCE LTD.) 18 May 1995 see the whole document ---	1-24
A	PEPTIDE, CHEMISTRY AND BIOLOGY. PROC. 12TH AM. PEPT. SYMP., CAMBRIDGE, MASS., JUNE 12-16, 1991, 1992 ESCOM, LEIDEN, pages 791-792, R G ALMQUIST ET AL. 'Development of peptidomimetic inhibitors of a newly isolated atrial peptide-degrading enzyme' see the whole document --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
12 January 1996	22.02.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Masturzo, P

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INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/GB 95/02362

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 104, no. 9, 3 March 1986 Columbus, Ohio, US; abstract no. 65259b, F FAHRENHOLZ ET AL. 'Photoaffinity labelling of the renal V2 vasopressin receptor. Identification and enrichment of a vasopressin-binding subunit' page 366; see abstract & EUR. J. BIOCHEM., vol. 152, no. 3, 1986 pages 589-595, ---	1
T	JOURNAL OF BIOLOGICAL CHEMISTRY (MICROFILMS), vol. 269, no. 48, 2 December 1994 MD US, pages 30227-30231, N FOTOUHI ET AL. 'Potent peptide inhibitors of stromelysion based on the prodomain region of matrix metalloproteinase' see the whole document and especially the compounds of Table VI -----	1-24

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INTERNATIONAL SEARCH REPORT

...rmation on patent family members

Interna...al Application No
PCT/GB 95/02362

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9513289	18-05-95	AU-B- 8113394	29-05-95

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